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TOXIC EFFECT OF BORON ON FRUIT TREES[1]

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(WITH THIRTEEN FIGURES)

It is now well established that small concentrations of boron are indispensable to the growth of certain, if not all plants, and that toxicity results when these small concentrations are slightly increased. Several investigators have recently reviewed the literature bearing on the relation of boron to the growth of plants, and therefore it will be unnecessary in this paper to enter into a discussion of the literature. KELLEY and BROWN (4) have shown that boron occurs in certain soils and irrigation waters of southern California in amounts sufficient to cause severe injury to citrus and walnut trees. The writer has made a study of the toxic effects of boron under controlled conditions, and has produced symptoms in the leaves of certain fruit trees similar to those found in certain orchards of California.

It is of interest at the outset to determine the lowest concentration of boron that will cause injury to citrus seedlings. When grown in the same culture jars, lemon seedlings were found to be more sensitive than orange seedlings to the effects of boron. Unless otherwise stated, boric acid was the source of boron used in all experiments. At a concentration of 1 p.p.m. of boron there was slight injury to all of the lemon leaves and the new leaves became yellow, while the orange leaves were unaffected. At a concentration of 2

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p.p.m. all of the lemon leaves showed injury but again the orange leaves showed no effects. Injury first became noticeable on orange leaves when the nutrient solution contained 3 p.p.m. of boron, and then only after the seedlings had been grown in the solution for at least 2 months with frequent renewal of the culture solution.

After 2 months' growth in Hoagland's solution to which boron was added, only 2 out of 25 sweet-orange seedlings showed injury at a concentration of 3 p.p.m. of boron, while with 6 p.p.m. every plant showed injury.

Sweet-orange and lemon seedlings were grown in the same culture jars. Hoagland's nutrient solution was used, to which 10 to 40 p.p.m. of boron was added in the form of manganese borate (MnB_4O_7). In every case injury was produced. Although manganese borate is usually considered to be insoluble, its low solubility is sufficient to produce injury in citrus trees. BRENCHLEY and WARINGTON (1) found that the addition of the so-called insoluble borates to culture solutions sufficed to supply boron in quantities adequate for growth.

The writer has grown lemon and Valencia orange trees budded on sour-orange stocks in dust and rain-protected sand cultures without the addition of boron, and after 3 years the leaves when analyzed by Mr. S. M. BROWN, Assistant Chemist in the Department of Agricultural Chemistry of this Station, showed about 50 p.p.m. of boron in the dry matter. KELLEY and BROWN (4) have shown this concentration to be typical of normal trees when grown in California. As the trees were planted bare-rooted, using only the larger roots and a portion of the trunk, it must be assumed that either sufficient boron gained entrance to the sand in the form of dust in spite of the protection, or that a small amount of comparatively insoluble borate occurred in the original sand and that this maintained a supply of boron adequate for the growth of leaves. A more successful method of reducing the content of boron in the trees may possibly be found by the use of water cultures extending over a period of years. Studies are now under way regarding methods of growing citrus for long periods in water culture, and these may afford suggestions in this direction.

A Eureka lemon and a Valencia orange tree, each budded on sour-orange roots, were grown for 3 years in tanks of sand 4 feet in diam-

eter and 5 feet deep, during which time they were supplied with Hoagland's culture solution. Each tree was irrigated with 432 liters of culture solution containing 25 p.p.m. of boron, and this was followed by Hoagland's solution without boron. The lemon tree was the first to show injury. The leaves burned along the margins and soon abscissed. The new leaves that developed later were very large, thin, and pale yellowish green along the outer portion, but a darker green near the midrib. They were also badly crinkled (fig. 1) and

FIG. 1.—Right, normal lemon shoot bearing healthy leaves; left, shoot from lemon tree in sand culture that received boron, bearing burned mature leaves and pale yellowish green and crinkled young leaves.

abscissed without becoming mature. The Valencia orange leaves were at first dark green, but, as a consequence of the boron treatment, they became somewhat mottled between the veins near the tip. The yellow areas enlarged, until the leaves finally became almost entirely chlorotic (fig. 2). These leaves fell prematurely. The succeeding leaves were thin in appearance and of a pale yellowish green color, but showed no tendency to crinkle, as did the lemon leaves. In this connection the results of WARINGTON (6) are of interest, in that she found boron injury to be accompanied by chlorosis.

The preceding results were confirmed by experiments with 2-year old, budded lemon trees grown in sand cultures in containers 20

inches in diameter and 26 inches deep. Hoagland's solution, to which 25 p.p.m. of boron was added, was used. The leaves became somewhat mottled and burned, and the new leaves were thin and crinkled, as in fig. 1.

When such cultures were washed free from the excessive boron before the injury became too pronounced and were then given unmodified Hoagland's solution, the new cycles

FIG. 2

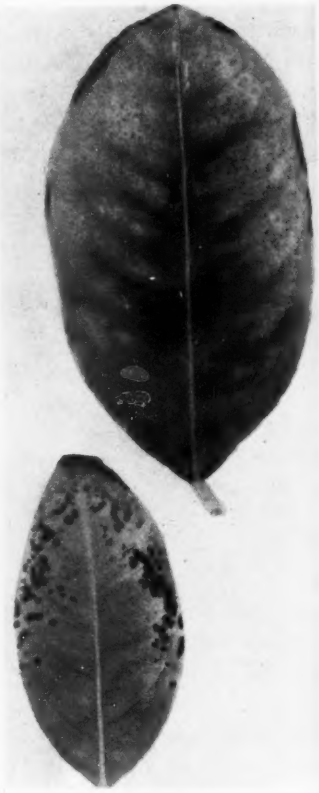

FIG. 3

FIG. 2.—Toxic effect of boron on leaves of Valencia orange tree grown in sand culture: advanced case of chlorosis with slight bronzed coloration of yellow areas.

FIG. 3.—Toxic effects of boron upon leaves of lemon trees in sand culture: upper, dorsal surface of leaf showing destruction of chlorophyll; lower, ventral surface of leaf showing dark, slightly elevated, resinous spots.

of growth were badly crinkled, but the later cycles were normal in appearance. These results, as well as those obtained in soil cultures, indicate that, unless the trees are badly injured by boron and have

been defoliated one or more times, they may recover after the toxic agent has been leached down below the root zone. The initial stages of the leaf injury were not accompanied by visible evidence of root injury. An examination of many sections of boron-affected leaves failed to show effects upon the vascular system such as WARINGTON (6) found with the broad bean when the supply of boron was inadequate.

The effects of boron on lemon trees depend not only on the concentration of boron in the culture solution, but also upon the nature of the culture solution. When grown as sand cultures with one application of 9 liters of Hoagland's solution containing 50 p.p.m. of boron, lemon leaves showed a rapid destruction of the chlorophyll and a marginal burning. These effects were accompanied by a type of mottle, and the production of numerous dark brown or black slightly elevated resinous spots on the ventral surface of the leaf, which can even be seen to some extent on the dorsal surface, as shown in fig. 3. Similar spots may also be produced by a high concentration of sulphate. If we apply Hoagland's solution, enriched with additional calcium nitrate and 100–400 p.p.m. of boron, to Valencia orange trees, the leaves may become dry and curl tightly prior to their early abscission, without going through the preliminary stages of toxicity. These lethal concentrations produced effects upon the leaves that closely resembled permanent wilting.

Since a large part of the inorganic constituents of citrus leaves consists of calcium, it is of interest to study the effects of a nutrient solution containing boron but deficient in calcium. Such cultural treatment brought about a spotting of the leaves as a result of the burning of small areas between the veins (fig. 4), and also caused premature abscission. This indicates that the leaf symptoms resulting from toxic concentrations of boron are dependent to some extent at least on the concentration of other ions. BRENCHLEY and WARINGTON (1) found an association between the presence of boron and the absorption of calcium in certain plants.

The effect produced by known concentrations of boron in soil cultures was next studied. Valencia orange trees were grown in 10-gallon earthenware jars filled with soil, to which was added 2 liters of Hoagland's solution containing sufficient boron to give concentra-

tions of 3, 6, 9, 12, and 15 p.p.m. of boron (in terms of dry soil). Control trees were also grown in soil which received the nutrient but no boron. All cultures then received 2 additional liters of Hoagland's solution. The drainage system of the crocks was controlled so that no solution escaped without being reapplied to the surface

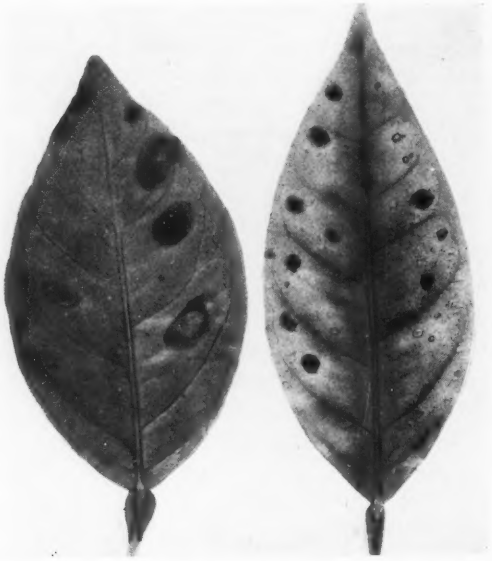

FIG. 4.—Toxic effects of boron on leaves of Valencia orange trees in sand cultures containing calcium carbonate as source of calcium; water-soluble calcium in cultures very low in relation to potassium.

of the soil. After 11 months all of the trees in the boron-treated soil cultures showed typical boron injury. Only a few of the oldest leaves of the tree growing in soil containing 3 p.p.m. of boron showed effects, and these were so slight that, unless one were aware of the boron additions to the soil, they might easily remain unobserved. Had this been the case it might have been concluded that either the soil had the capacity to remove boron permanently from the field of root absorption, or that the supply of boron added to the soil was

insufficient to become an excess in the large tree top. The oldest leaves are the first to show an excess of boron.

The later cycles of growth of the trees, which were grown in soil containing 3 p.p.m. of boron, were normal in appearance. With a

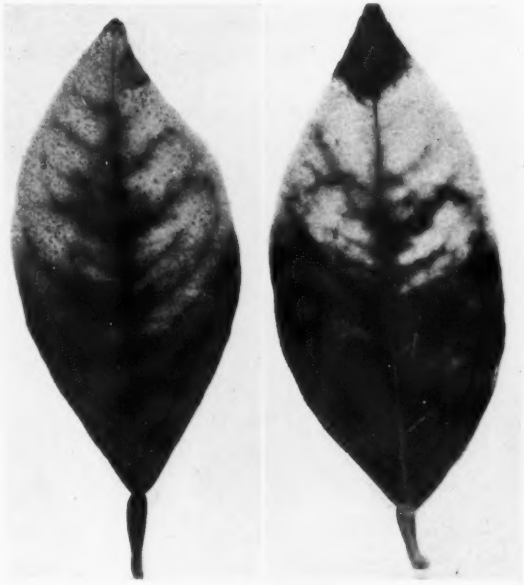

FIG. 5.—Toxic effects of boron on leaves of Valencia orange trees grown in soil cultures: left, ventral surface of leaf; right, dorsal surface of leaf; chlorosis progresses toward base of leaf blade, followed by burning of leaf tip and slightly bronzed coloration of chlorotic portion.

concentration of 6 p.p.m. or more of boron, the trees showed increased injury, as shown in fig. 5. The apical portion of the leaves was the first to show yellowing of the chlorophyll. As the leaves increased in age the yellow portion took on a bronzed appearance and developed tip burn. Gradually the chlorosis extended downward toward the basal end, the dark green color persisting longest near the larger veins. The effects are distinguishable in most cases from true mottle-leaf in that the terminal half of the leaf appeared to be af-

fected more than the basal half, and the tips and margins as a rule usually became brown.

The severity of the injury brought about by the addition of a given amount of boron decreases as the trees put on new cycles of growth. This is because the supply of boron is partially exhausted by the earlier growth. This fact indicates that there is very little if any migration of boron from the mature leaves out into the new growth;

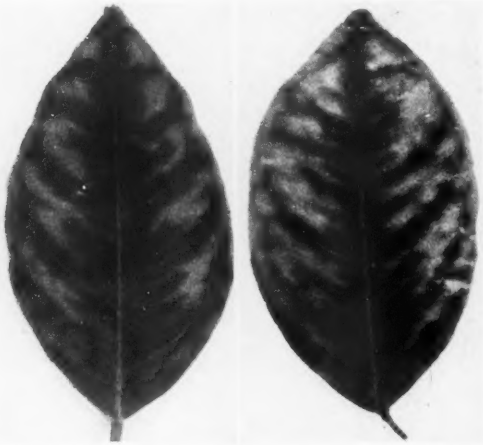

FIG. 6.—Effect of boron on mottling of leaves of Valencia orange trees in soil cultures that received sodium nitrate: left, slightly mottled leaf from cultures that received no boron; right, severely mottled leaf from cultures that received boron.

consequently the amount of boron required to produce injury is dependent on the size of the tree. Since in the state of nature many of the abscissed leaves are blown away, and soluble boron can be leached out of the soil, it is possible that the concentration of boron in the soil tends to be held in check by purely natural causes.

Under certain limited conditions the effect produced by boron may be not very different from that of true mottle. The Valencia orange leaf shown to the left in fig. 6 is typical of leaves grown in soil cultures receiving nitrate of soda. When small amounts of boron were added along with the nitrate of soda the mottle was intensified (fig. 6).

Lemon trees in soil cultures that received 5–10 p.p.m. of boron whenever irrigated, showed the boron effects seen in fig. 7. The leaves show marginal and tip burn, and the yellow areas between the veins also show burn. The resinous spots on the ventral surface of the leaf follow the progress of the chlorosis. Fig. 8 shows the effects of boron upon grapefruit leaves.

Lemon seedlings were grown in water cultures which received Hoagland's solution plus 7.5 p.p.m. of boron and varying amounts of iron sulphate. The ferric salt was used instead of the ferrous salt, in view of the statement (COMEY 3) that water may dissolve out all of the boric acid from ferrous borate but that ferric borate is insoluble in water. Fig. 9 shows the results obtained. All of the cultures received small amounts of ferric citrate from time to time.

The reduced growth at the higher concentrations of iron may possibly be related to the increased acidity of the iron solution, as well as to the excessive amounts of iron. There can be no doubt that the iron was effective in overcoming the toxic action of the boron. The growth obtained may have resulted either from the catalytic action of the iron or from the precipitation of the boron as insoluble ferric borate. BRENCHLEY and WARINGTON (1) have made the important observation that toxicity results from boron if iron is left out of the cultures.

The effect of other salts on the toxicity of boron was also studied. Among these aluminum sulphate, lead chloride, chromium nitrate, and zinc chloride may be mentioned. Of these salts only aluminum sulphate at concentrations up to 40 p.p.m. and lead chloride at concentrations from 65 to 170 p.p.m. appeared to give slightly better growth than the controls.

Boron toxicity was next studied with walnut seedlings grown in Hoagland's solution. Boric acid was added to some of the cultures in amounts ranging from 2.25 to 22.5 p.p.m. of boron. After 7 weeks the leaves of the plants which were grown in the solution containing 6.75 p.p.m. of boron showed curling and burning at the margin. At higher concentrations the leaves were curled and burned more severely, with a slight mottle occurring at the highest concentration.

In order to study the effect of concentration of boron on growth, several species of fruit trees were grown as sand cultures. The con-

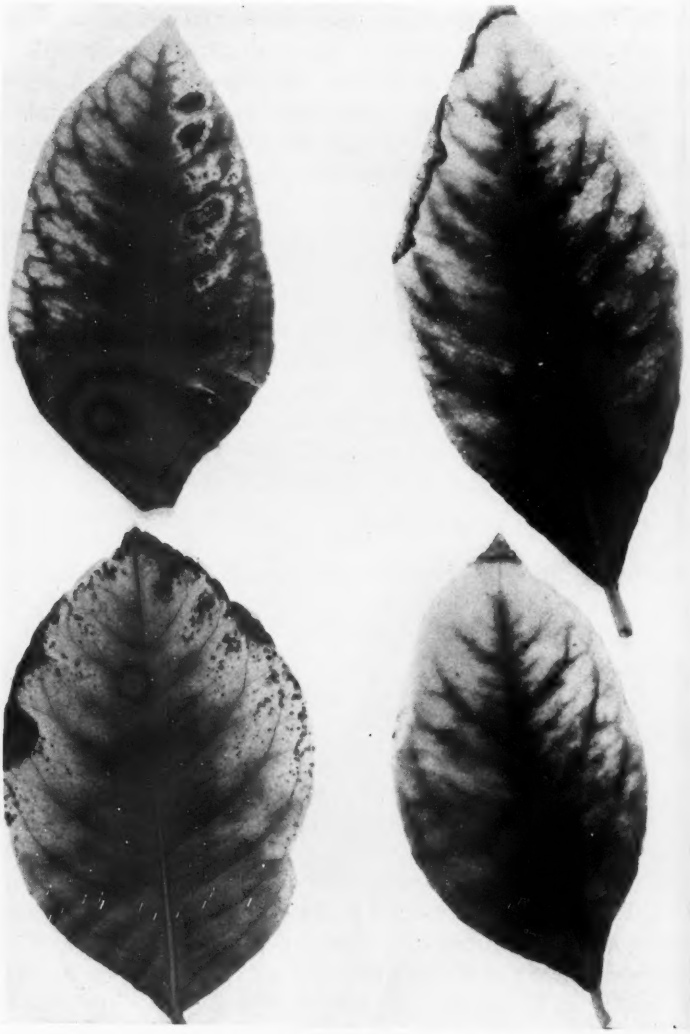

FIG. 7.—Toxic effects of boron on leaves of lemon trees in soil cultures: lower left, ventral surface of leaf showing resinous spots and marginal burning; lower right, leaf chlorotic with tip burn; upper right, further progress of chlorosis from leaf tip, tip burn, and marginal burning; upper left, burning of chlorotic areas and appearance of bronzed effect between veins.

tainers were 20 inches in diameter by 26 inches deep. The containers were divided into three groups. The first group consisted of containers planted to Fuerte avocado (one), Eureka lemon (two), and Valencia orange (two); the second group consisted of containers

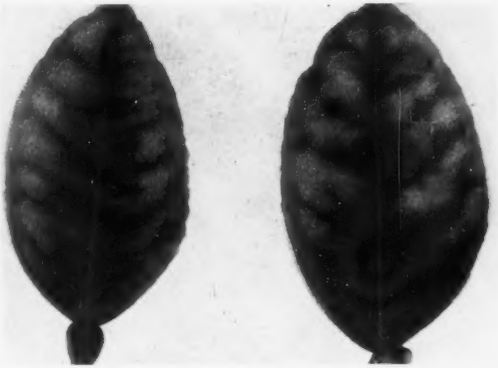

FIG. 8.—Toxic effects of boron on leaves of grapefruit trees in sand culture

FIG. 9.—Effects of ferric sulphate on growth of lemon seedlings in Hoagland's solution containing 7.5 p.p.m. of boron; concentration of iron as ferric sulphate added: left to right, 5, 10, 15, 25, 40, 65, 105, 0, 0 p.p.m.

planted to Stewart pecan (one), Pomelo (one), *Juglans regia* (one), Fuerte avocado (one), Elberta peach (two), and Eureka lemon (one); the third group consisted of containers planted to three to nine trees of each variety. The three groups of containers received half strength Hoagland's solution, with the addition of 1 p.p.m. of boron to the first group, 2 p.p.m. of boron to the second group, and no boron to the third group. Tap water was used in preparing the 50-gallon barrels of culture solution. The solutions were thoroughly mixed and

run in a fine continuous drip into the containers. The amounts of solution applied were considerably in excess of the transpiration requirements of the trees. In this way the desired concentration of boron in the sand cultures was maintained at a fairly constant level.

The experiment was begun in March, and in the following December leaf samples were taken for boron analysis. With concentrations of 1 or 2 p.p.m. of boron, the avocado leaves showed burned spots scattered throughout the entire leaf surface, but most conspicuous along the margin. These spots coalesced as the injury proceeded. When the culture solution contained 2 p.p.m. of boron the pecan leaves burned badly along the margin, and the new leaves were curled somewhat, which gave them a crinkled appearance. This condition of the avocado and pecan leaves is evident from fig. 10. Fig. 11 illustrates the effect of boron accumulation in peach leaves when the culture solution contains 2 p.p.m. of boron. The leaves burned severely along the entire margins and became wrinkled, later splitting as the drying out of the margins increased.

The leaves of the walnut tree in the culture that received 2 p.p.m. of boron showed burning in spots along the leaf margin. These coalesced, and finally the entire leaf margin became involved, as shown in fig. 12. At the same time many of the leaves became yellow between the veins, and subsequently they burned in these mottled areas as well as along the margin, as will be shown later in another connection. WARINGTON (5) found that boron injury with barley was characterized by a spotting of the leaves, while in the case of the broad bean there was a brown band along the margins. A spotting with burned areas on soy bean leaves has been produced with boron by COLLINGS (2).

In the sand cultures irrigated with a solution containing 2 p.p.m. of boron, the oldest grapefruit leaves showed boron injury only to a minor extent. The new growth was extremely vigorous, although toward maturity these leaves became somewhat pale in color and showed slight injury.

All of the mature leaves of the lemon tree treated with 2 p.p.m. of boron showed severe boron injury. The new leaves developed to full size but only the lowest ones have as yet shown any injury. The young leaves may make excellent growth for a time, only to be in-

jured later. Since maturity of the leaves is greater the farther they occur from the shoot terminus, the injury due to boron is likewise greater in the same direction.

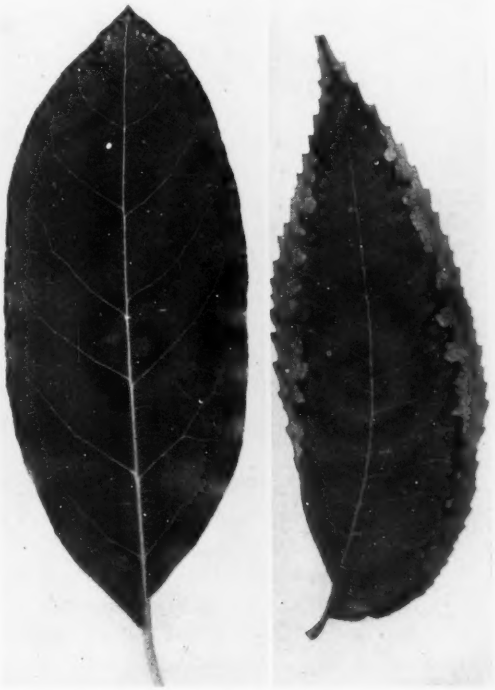

FIG. 10.—Toxic effects of boron on leaves of avocado and pecan trees in sand cultures: left, avocado leaf with dead spots scattered throughout and joining along edge to give appearance of marginal burning; right, pecan leaf with severe marginal burning.

The lemon and orange trees that received a culture solution containing 1 p.p.m. of boron showed no injurious effects. These trees grew most vigorously, and all of the growth was healthy in appearance. The orange trees made less growth than the lemon trees but the growth was normal in appearance. Leaf samples collected from these trees were analyzed for their boron content by MR. S. M.

BROWN. Table I gives the concentration of boron in the leaves of trees grown under controlled conditions, as compared with that of leaves from trees grown in the field.

The concentration of boron in the avocado leaves was not very high even in the affected leaves, although the amount present was relatively high when compared with that in normal leaves. The grapefruit leaves contained the largest amount of boron and still were not badly affected. Wide variation may exist in the boron content of leaves that do not show any symptoms. Lemon and orange leaves were found to contain over 100 p.p.m. of boron when only 1 p.p.m. was contained in their culture solution, and, notwithstanding this concentration of boron in the leaves, they still possessed a healthy appearance. When 1 p.p.m. of boron occurs in an irrigation water in the field, the concentration of boron in the soil solution is subject to considerable fluctuation as a result of such factors as evaporation of water from the soil, excessive irrigation, and frequency and amount of rain. As a consequence of these factors operating in the field, which have been described by KELLEY and BROWN (4), injurious concentrations of boron may be reached in the soil for the growth of citrus, a condition that was obviated in these controlled sand cultures.

FIG. 11.—Toxic effects of boron upon leaves of peach trees in sand cultures.

Four-year-old walnut trees growing in the field at the Citrus Experiment Station were basined on September 27, 1926, and borax was applied to the soil. Two basins received 50 gm. each of c.p. powdered borax, two received 100 gm. each, two received 200 gm. each, and two received 400 gm. each. Adjacent trees served as controls. Only one application of borax was made.

On December 7, 1928, the trees in the basins that received 50 gm. of borax showed a slight trace of injury. Some of the leaves of the trees in the basins where 100 gm. of borax was applied became

slightly chlorotic and mottled, but did not burn. At higher concentrations of borax the leaves mottled badly and burned between the veins. Many were badly crinkled and burned along the margins, as shown in fig. 13. When 200 and 400 gm. of borax were applied to

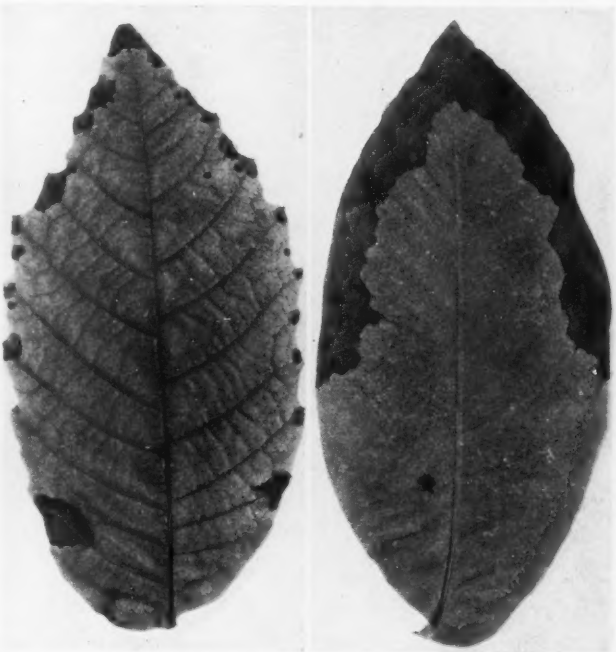

FIG. 12.—Toxic effects of boron on leaflets of walnut trees in sand culture: left, beginning stage of visible injury; right, advanced stage or marginal burning; mottle in both leaves screened out in making photograph.

the basins, the leaves of the second cycle of growth became full sized and were normal for a time; later they showed severe injury. The injury may not develop until after a considerable lapse of time, therefore, indicating a gradual accumulation of boron in the leaves. Boron determinations were made on samples of these leaves, the results of which are given in table II. The mottle and burning produced as a result of the borax applications resemble closely the condition of the leaves of many walnut trees in the field.

The injurious effects of boron on the mineral nutrition of leaves are of considerable interest. BRENCHLEY and WARINGTON (1) have found evidence of a definite association between boron and the absorption or utilization of calcium. In the absence of boron in the nutrient solution the calcium was not fully utilized. In the present studies we are considering toxic concentrations rather than minimal requirements for boron, and therefore it is of interest to investigate

TABLE I

BORON CONTENT OF LEAVES OF TREES GROWN IN SAND CULTURES AS COMPARED WITH THAT OF LEAVES OF HEALTHY TREES IN THE FIELD

TREE	LOCATION	CONDITION	BORON IN CULTURE SOLUTION (P.P.M.)	BORON IN DRY MATTER OF LEAVES (P.P.M.)
Avocado.....	Field, Box Springs Station	Healthy	.....	20
Avocado.....	Sand culture	Affected	1	70
Avocado.....	Sand culture	Affected	2	82
Pecan.....	Field, Riverside	Healthy	.....	24
Pecan.....	Sand culture	Affected	2	305
Walnut.....	Sand culture	Affected	2	218
Grapefruit.....	Plot U, Riverside	Affected	.....	34
Grapefruit.....	Sand culture	Slightly affected	2	480
Lemon.....	Field, Box Springs Station	Healthy	.....	29
Lemon.....	Sand culture	Healthy	1	114
Lemon.....	Sand culture	Affected	2	322
Lemon.....	Sand culture	Full size healthy new leaves	2	54
Valencia.....	Field, Irvine	Healthy	.....	24
Valencia.....	Sand culture	Healthy	1	120

the effects of toxic concentrations of boron on the calcium content of the leaves.

The ash of orange and lemon leaves affected with boron was found to contain about 24 to 29 per cent of calcium and about 10 to 14 per cent of potassium. The ash of mature healthy orange and lemon leaves usually contains from 30 to 35 per cent of calcium and from 4 to 6 per cent of potassium. Citrus leaves injured by excessive boron therefore have a reduced content of calcium and an increased content of potassium. This type of composition corresponds to that of leaves that are mottled or immature. The excessive boron appears to poison the leaf tissues and to interfere with the absorption of calcium.

Samples of walnut leaves taken on September 26, 1927, showed the ash of the leaves from the trees where the largest application of borax was made, as containing 19.90 per cent of calcium and 12.52 per cent of potassium; while that of the leaves of control trees showed 24.71 per cent of calcium and 9.55 per cent of potassium. The ash composition of the leaves of these borax-treated walnut trees resembles that of mottled or immature walnut leaves. The ash of the water-soluble fraction of the dry matter of affected leaves was 27.74 per cent of the total ash, compared with 24.24 per cent for control leaves. The water-soluble calcium of the dry matter as a percentage of the total calcium was 4.44 per cent for affected leaves and 2.08 per cent for leaves of control trees.

FIG. 13.—Toxic effects of single application of borax added to soil, on leaves of walnut trees in field; lower left leaf shows mottling; upper right leaf shows mottling to point of chlorosis and also marginal burning.

The soluble potassium was 86.84 per cent of the total potassium in the dry matter of the affected leaves while that in control leaves was 91.53 per cent. The total magnesium in the ash of affected leaves was 4.79 per cent while that in leaves of control trees was 6.25 per cent. The soluble magnesium expressed as a percentage of the ash of the soluble frac-

tion was 6.33 per cent and as a percentage of the total magnesium was 36.61 per cent; while the values for control leaves were 13.64 and 52.83 per cent respectively. Leaf samples were taken from all of the

TABLE II

BORON IN LEAVES OF WALNUT TREES THAT RECEIVED ONE APPLICATION OF BORAX

APPLICATION (GM.)	BORON IN DRY MATTER OF LEAVES (P.P.M.)
50	105
100	143
200	192
400	360

trees again on December 7 and their analyses confirm these earlier results. An excess of boron therefore may disturb the mineral nutrition of leaves, and frequently so seriously that, as a result of the reduced calcium content, the leaves absciss prematurely.

Summary

1. Boron is toxic to plants when present in relatively small concentrations.

2. Lemon seedlings are more sensitive to boron than orange seedlings. The new growth of seedlings affected with boron may be chlorotic.

3. By the continuous flow method of supplying the culture solution, the relationship was studied between the concentration of boron in the culture solution, the effect on the tree growth, and the concentration of boron in the leaves.

4. The effect of boron on citrus depends on the concentration of boron present in the nutrient solution and the concentration of other nutrient ions.

5. Additions of boron greatly intensified the mottling tendency of the Valencia orange trees when grown in soil treated with nitrate of soda.

6. Citrus and walnut leaves may become thin, mottled, chlorotic, and crinkled as a result of a toxic agent such as boron.

7. The addition of various amounts of ferric sulphate to cultures of lemon seedlings tended to overcome the toxicity of boron. So-

called insoluble borates in water cultures may furnish low concentrations of boron to the plant roots, but the continued absorption of these small amounts may be sufficient to produce toxicity. Several so-called insoluble borates were found to be distinctly toxic.

8. Unless badly defoliated repeatedly, trees injured by boron may recover if the toxic agent is leached out with water.

9. With toxic concentrations of boron, no changes in the vascular anatomy of the affected leaves were observed such as have been reported when boron was absent.

10. A single application of borax, ranging from 50 to 400 gm. per tree, to basined soil in which walnut trees were growing in the field produced a mottle and a leaf burn chiefly along the margins.

11. Citrus and walnut leaves affected with boron contain reduced amounts of calcium and increased amounts of potassium. The composition is typical of mottled and of immature leaves. The leaves, it is believed, are prevented from becoming mature in regard to ash composition as a consequence of the paralyzing action on the growth processes.

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CYTOLOGICAL STUDIES IN CYPERUS, ELEOCHARIS, DULICHIUM, AND ERIOPHORUM[1]

G. CLAUDE HICKS

(WITH PLATES VIII, IX)

The presence of aneuploidy has been demonstrated in the Cyperaceae by two European investigators, in *Carex* by HEILBORN (**11**, **12**, **13**) and in *Scirpus* by HAKANNSON (**10**). In a previous paper, the presence of aneuploidy has been described in American species of *Scirpus* (**14**), and evidence adduced in favor of a hybrid hypothesis as an explanation of this condition. The present study deals with the chromosome conditions found in *Dulichium*, *Cyperus*, *Eriophorum*, and *Eleocharis*.

Materials and methods

The material was collected as it grew in the environs of metropolitan Boston and other points near the Atlantic seaboard. The Gray Herbarium provided facilities for check work in the matter of plant determinations.

The inflorescences were collected on warm days and put immediately into Carnoy's fluid. The air was then drawn quickly from the tissues by means of an exhaust pump to insure rapid fixation. Material gathered in the early afternoon gave more satisfactory results on the whole. After being left in the fixative for 24 hours, the plants were washed in from two to four changes of 95 per cent alcohol.

It was generally found necessary to demineralize and bleach. A stock solution for such was made by adding crystals of sodium chlorate to strong hydrofluoric acid until a saturated solution was obtained. The material was passed into water and then put into wax or wax-coated bottles, containing a dilution of the fluid described, and left for only such time as was necessary for the required degree of softening and bleaching. Following this treatment the material was dehydrated and imbedded in nitrocellulose. A Jung-Thoma slid-

[1] Contributions from the Laboratories of Plant Morphology, Harvard University.

ing microtome was used for cutting sections. Both longitudinal and transverse sections were cut 10 and 5 μ thick. It was demonstrated that transverse sections were the best for details.

Haidenhain's iron-haematoxylin was used for staining, and gave satisfactory results. A Bausch and Lomb microscope equipped with a 140° Abbé condenser was used. Since strong light and resolution were very important factors, a 3 mm. 140° Bausch and Lomb apochromatic lens, together with a no. 15 periplan compensating ocular, provided an excellent combination of equipment. The drawings were made with the aid of a camera lucida and then enlarged three times.

Counts of the chromosomes were made mostly from the heterotypic metaphase plates, and where possible use was made of diakinetic and homeotypic stages.

The chromosomes in *Cyperus* are rather small, and it is difficult to know the exact conditions of affinity from a study of diakinesis. The other genera, particularly *Eleocharis*, display almost diagrammatic conditions. It has seemed best not to group the chromosomes into classes as no consistent results are obtained upon the whole. Haploid counts of the chromosomes are recorded.

CYPERUS

Cyperus dentatus Torr.—Material from Long Pond, Natick, Massachusetts, shows 17 chromosomes (fig. 3), one of which is conspicuously larger than the others. The chromosomes behaved normally in all the divisions forming the regular four nuclei, three of which aborted in the usual manner. The spikelets of this species are often abortive and are changed into leafy tufts, even though the pollen is rather well formed up to the time of shedding.

Cyperus sp.—This species, collected by JEFFREY in New Zealand, could not be identified as the material was not mature enough. The spikelets are rather broad, and there is one anther only to a flower. According to CHEESEMAN (6) it may be *C. tenellus* L. It would appear that all the chromosomes are bivalents. The metaphase plates (fig. 4) always show 21 chromosomes, two of which are conspicuously larger than the rest, 7 are smaller, and 12 are of medium size. The large chromosomes not infrequently show a twofold nature as if

they were quadrivalents, and but very little material staining darkly is found in the cytoplasm.

Cyperus erythrorhizos Muhl.—Plants were collected at Winter Pond, Winchester, Massachusetts. The chromosomes are disorderly in arranging themselves at the metaphase plate (fig. 5). The 48 elements seen are much smaller than those found in diakinesis. This difference may be due to clumping or may be due to weakened affinity. Interkinesis is apparently of short duration. The surviving nucleus divides very promptly after the abortive nuclei have begun their degeneration.

Cyperus esculentus L.—Material found at Jamaica Pond, Boston, proved suitable for study. Fig. 6 shows a heterotypic plate. While at times there is a grouping of the chromosomes so that accurate counting cannot be made, there are also good clear plates which possess 54 chromosomes of various sizes. All stages of division are regular.

Cyperus filiculmis Vahl. var. *macilentus* Fern.—Collections of this species that proved of value were made at South Sudbury station and at Sharon, Massachusetts. The anther sacs are extremely small, and as the pollen mother cells are not more than 12 cells in height, much material had to be sectioned to provide a basis for accurate results.

Diakinesis stages show larger chromosomal elements than the metaphase plates. It is in the movement of the chromosomes from the metaphase plate that one comes upon irregularities not unlike those found in the irregular species of *Scirpus americanus* (**14**) found at Hyannis, Massachusetts. Fig. 8 shows this condition. Fig. 7 shows a metaphase plate. There are 73 chromosomes which are exceedingly difficult to delimit. This number cannot be considered as final, for not enough clear plates have been available. The homeotypic divisions seem to be regular, and cell plates are found in both divisions.

Cyperus strigosus L. var. *compositus* Britton and *C. ferax* Rich.—These have been found to have very large chromosome numbers, but the non-availability of good clear figures, as noted in *C. filiculmis* var. *macilentus*, has only provided inconsistent and unreliable results. The former is open to the suspicion that it is heterozygous material.

Eriophorum

Eriophorum virginicum L.—Fig. 2 shows good clear plates from plants collected at Tewkesbury, Massachusetts. Twenty-nine chromosomes have been found to be present: 2 larger, 8 smaller, and 19 of medium size. It will be seen that the chromosomes are split in preparation for the homeotypic. All stages have been found to be regular.

Eriophorum gracile Roth. and *Eriophorum tenellum* Nutt.—These showed only mature pollen.

Dulichium

Dulichium arundinaceum (L.) Britton.—Material of this species was gathered at Wakefield, West Manchester, and Bedford, Massachusetts. Of all the species in the present connection this was the most difficult to differentiate, as the cytoplasm possessed a very "muddy" appearance. No stages of diakinesis have been studied satisfactorily. The anthers are exceedingly long, and starting at the top of the anther sac with the heterotypic metaphase, we find all transitions, to the late anaphase at the bottom. Both divisions are regular, and pollen formation is normal. In all cases, 16 chromosomes (fig. 1) have been found.

Eleocharis

Eleocharis obtusa (Willd.) Schultes.—This is recorded as being "very variable in size and habit" (**24**). Several collections were made at Moncton, N.B., Canada, one at Glenwood, Massachusetts, and another was kindly sent by Dr. A. E. Longley from Washington, D.C. This species may be most easily confused in the field with *E. ovata* (Roth.) R. & S. Accordingly, material was collected only from clumps in which plants showed the "tam o' shanter" cap on the achenes. This is possible because both flowering and mature material are found in the same clump.

The haploid number is shown to be 5 large and equal chromosomes, which is low. The behavior in all regions is practically the same. Fig. 9 shows a late diakinesis, 4 of the chromosomes having lost the bivalent configuration which has been retained by the fifth.

This might also be interpreted as the cause of the slight lagging sometimes observed.

Dark material is seen in the cytoplasm. The heterotypic metaphase is quite orderly. The spindle fibers end in a point, a rather exceptional condition.

The plants from Washington were the only material to show any irregularity in the heterotypic metaphase. These were very tall. In the homeotypic anaphase, however, there were in addition to the fairly regular figures, those in which the chromatin was strung out along the fibers, and it was impossible to delimit the chromosomes. Four nuclei were always seen upon the termination of the divisions.

The pollen at the time of shedding is very much shrunken, although before that time it appears quite normal.

Eleocharis sp.—An unidentified species was collected by JEFFREY in New Zealand, and it is interesting from the standpoint of chromosome number. In all stages (diakinesis etc.) there are present 10 chromosomes. The divisions are regular. One large chromosome is conspicuous from the others (fig. 10).

Eleocharis tuberculosa (Michx.) R. & S.—A single collection of this plant was made in a wet region in the Concord River meadows at Bedford, Massachusetts, and the preservation of the material was very poor. The metaphase plates were for the most part hopelessly clumped together, and a suitable study of diakinesis was impossible because of cytomixis.

In several anther sacs, clear plates of the heterotypic metaphase possessed 15 chromosomes (fig. 11), which may be divided into classes of average size, 7 larger and 8 smaller. While the chromosomes could not be checked in their behavior, from the gross appearance meiosis was quite normal and 4 nuclei only were formed. The pollen was somewhat shrunken at the time of shedding, but there were always considerable protoplasmic contents in the cells.

Eleocharis capitata (L.) R. Br.—The nomenclature of this species has been revised by BLAKE (4): it is the *E. tenuis* (Willd.) Schultes of GRAY'S manual. Material from Chestnut Hill, Massachusetts, and from Wolfville, N.S., Canada, which Professor R. H. WETMORE kindly collected for me, showed similar conditions.

Size differences are seen in the polar views of the metaphase

plates (fig. 12). In diakinesis there is a quadrivalent arrangement of two of the chromosomes. This affinity at other times forms a large mass which appears to be made up of more than four elements. This quadrivalent formation must be only transitory, however, since it has never appeared in the metaphase plates. The nucleolus possesses a scalloped appearance and it is soon to disappear. Its degeneration is marked first by small vacuoles or areas that do not take the haematoxylin stain. These vacuoles soon coalesce, and only the border retains the normal dark blue color. Later on the whole nucleolus fades and is no longer seen.

The size differences of the chromosomes are seen to be of two average classes, 4 larger and 15 smaller. In the upper left part of the pollen mother cell is a black mass. This is one of the larger chromosomes passing over in cytomixis from the metaphase plate of an adjoining mother cell. The heterotypic anaphase is normal; the state of interkinesis is of some duration, and in this stage nucleoli are very seldom apparent. In the homeotypic anaphase the size differences are again seen. The abortion of the degenerating nuclei is entirely normal.

Eleocharis palustris (L.) R. & S.—In GRAY'S manual, seventh edition, this species is cited as "very common and very variable either in water, where it is rather stout and tall, or in wet grassy grounds, where it is slender and lower."

A comprehensive study of this species was made, and the results show from a morphological point of view that the species is really a group. Counts were made in diakinesis and in the heterotypic and homeotypic metaphases. It was demonstrated clearly that only the early stages of diakinesis were of value; then the pairing chromosomes from Y's, O's, X's, etc. Soon each of the bivalent masses gives way to a rounded mass, and the later stages are no longer of value and much more difficult to study than the metaphase plates. The slender and lower types of the species mentioned showed the haploid number to be 8. Fig. 13 shows the condition in plants growing along the wet shores of Lake Waban, Wellesley, Massachusetts. While it is to be remembered that chromosome size appears to be largely a matter of averages, it is obvious that there are size differences in the chromosomes: 2 more massive, 1 medium, and 5

smaller. The divisions are quite regular, excepting that soon after diakinesis dark material is seen in the cytoplasm. It will be noted that a constriction occurs in the chromosomes of the heterotypic metaphase plates (figs. 13, 15). This appearance has been noticed in wheat and other material, and described as a preparation for the homeotypic divisions. The condition here persisted for a surprisingly long time in interkinesis and even in the aborting nuclei. This same split condition occurs in the divisions of the embryo sac mother cell.

In members of the species collected at the head of Spot Pond and at a pond locally called Frog Pond in Chestnut Hill, however, there appeared occasionally in the metaphase plates 9 chromosomes in place of the usual 8. Fig. 14 shows such a condition. This appearance is probably due to the failure of the bivalents to pair. In anther sacs with this abnormal number, the chromosomes at times showed a lack of regularity. Fig. 16 shows lagging of the chromosomes in the anaphase. At the left it would appear that non-disjunction might take place. While the twofold nature of the chromosomes is often confusing, at the end of the heterotypic anaphase several clear figures have shown more than the normal elements. Counts of the young pollen grains, however, have never shown more than 8 chromosomes.

More interesting still are the variants of the stout plants growing in the water at Heard's Pond, Wayland, Massachusetts. There diakinesis often shows the bivalent pairs rather well, and conspicuous among the contents is always a "pair" which is not to be taken for a tetrad. Its behavior shows its elements to be quadrivalent in nature, and the halves are found not only lying side by side but also end to end. The metaphase plate (fig. 15) shows 18 chromosomes, 5 larger and 13 smaller of average sizes. Of the 5, two usually form the pair just mentioned, and a further pair may be formed by two of the remaining elements. Fig. 17 shows a profile of the heterotypic metaphase, and foremost among the elements there is a large quadrivalent chromosome. It may be said in passing that such structures as these have been found in tetraploid plants.

The heterotypic anaphase has never shown any irregularities. In the case of the homeotypic division (fig. 18) we find a distinct lagging, however, and conditions quite similar to those in fig. 16.

It would seem at times that the chromosomes failed to separate. Countings of the chromosomes in the young pollen grains, at the present time, seem to indicate that such happens. The pollen grains and pollen mother cells are much larger in the stouter form.

Eleocharis palustris (L.) R. & S. var. *glaucescens* (Willd.) Gray.—Only one of the collections proved to be of this variety. This was made at one of the smaller ponds near Fresh Pond, Cambridge. The homeotypic anaphase is regular, as were all the stages seen. In no case did there appear more than 4 nuclei in a pollen mother cell, contrary to the report of WILLE (**33**) in *Eleocharis palustris*, where 3 smaller and 2 larger ones were seen. In the larger forms growing at Wayland and Bedford, apparently every little seed was set, although the pollen was uniformly good before the time of shedding.

Eleocharis acicularis (L.) R. & S.—Members of this extremely small sedge were gathered at Heard's Pond, Wayland, Massachusetts, and at a small pond by the side of the road that runs past the Belmont Country Club.

The inflorescences suitable for cytological study are about 2 mm. long by 1 mm. broad, so that the anthers are extremely small and a great amount of material has to be cut to obtain the right stages. The material from Belmont showed the clearest results. The late prophase reveals, in the majority of cases, 28 chromosomes arranged in varying affinity. For the most part they are found linked in threes, but there are also to be found fours, twos, and ones. A like situation occurs in the metaphase plates (figs. 19, 20, 21). At times the chromosomes also appear as if in a serial arrangement, as described in *Carex aquatilis* by STOUT (**28**).

The number of chromosomes found in an inspection of the metaphase plates is a varying and puzzling one. In fig. 19 there are 26 of varying sizes, a smaller chromosome (chromomere?) being conspicuous. In figs. 20 and 21 are 29 chromosomes, but the sizes are different. These conditions are particularly puzzling as they may be found in anther sacs of the same plant. The chromosome numbers in these plates range from 25 to 29. Some of the differences may be explained in part by the fact that a group of the chromosomes may become attached longitudinally along the fibers of the spindle. Another feature is the lack of synchronization of chromosome move-

ment, for in one and the same anther sac there may be observed the heterotypic metaphase, heterotypic anaphase, and homeotypic metaphase.

The early anaphase of the heterotypic is always extremely irregular (fig. 22), with tangled figures, the elements of which are extremely difficult to delimit. The lagging of the chromosomes is observed in the late anaphase, but there have been seen no cases of chromosomes being left out in the cytoplasm.

Figs. 23 and 24 show the conditions found in the homeotypic metaphase plates. Counts vary, 18, 19, etc. Some of the trivalents have become oriented longitudinally on the spindle (fig. 24), and are disjoined in this division. Never has the same number of chromosomes been recorded as in the heterotypic plates.

In the anaphase of the homeotypic division there is extreme irregularity in the movement of the chromosomes. The elements of trivalent chromosomes go two to one pole and the remaining part to the other pole. At maturity there are often to be found anther sacs of shriveled pollen. Counts in the surviving tetrads have been made and are 15, 16, and 17. To establish the exact number, clear somatic plates unavailable at the present time will have to be examined. The material provided exceptionally clear and diagrammatic stages in the development of the embryo sac mother cell.

Discussion

When plants and animals are characterized by polymorphy, the behavior of the chromosomes in meiosis, and the quality of the reproductive bodies accompanying such diversity of form, are apparently characteristic. The evidence that polymorphy is very often seemingly due to a heterozygotic condition is now abundant and well known. It is conceivable that hybrid plants may not be abnormal in their reproductive behavior. If two plants closely enough related to one another are crossed, disturbances may not be more obvious than in pure strains. This has been demonstrated in known hybrids. It is also not a foregone conclusion that because a plant is of heterozygous origin it will be polymorphic. Constant hybrid blends are numerous and need no recital.

It will be the aim of this discussion to point out just how far we

may interpret the abnormal phenomena here, in the light of hybridization.

The natural hybrid *Drosera obovata* investigated by ROSENBERG (**25, 26**) presents a clear case of meiotic irregularities caused by univalent chromosomes. This species has as parents *D. longifolia* (20×) and *D. rotundifolia* (10×); consequently there are present 10 bivalents plus 10 univalents. The univalents are frequently left out in the cytoplasm, where they form small nuclei, and in the second division may develop small spindles. Disorganization of the pollen grains follows.

WODSEDALEK (**35**) has investigated the causes of sterility in the mule. There are no cases of its being interfertile. The mitoses found in the spermatogonial (somatic) cells are quite normal, but in the late prophase of the primary spermatocyte, the pairing of the chromosomes is never complete and is very inconsistent, as seen in the number of the chromosomes present. These range from 34 to 49, as compared with the 59 found in the spermatogonial cells.

The roses have long been distinguished by their polymorphy, and the cause was unknown or only suspected. The work of TÄCKHOLM (**30**) and of BLACKBURN and HARRISON (**2**) has demonstrated the concomitant cytological conditions. TÄCKHOLM divides this tremendous genus into three great classes: (1) those characterized by the occurrence of only paired chromosomes; (2) the very polymorphic Canina section in which bivalents and univalents are found usually in multiples of 7; and (3) aneuploid forms in which bivalents and univalents do not form multiples of 7. The first group contains diploid, tetraploid, hexaploid, and octoploid species and hybrids. The hybrids include many forms that are regular in the reproductive divisions and in spore formation. The second group, because they are always characterized by the presence of bivalents and of univalents, are analogous to the *Drosera* scheme of hybrids, and are considered by TÄCKHOLM to be F_1 generations. They are known for their widespread sterility, and have maintained themselves by apomixis. The Canina group by their crossing have given rise to the third group, which has irregular chromosome numbers because of irregularities in the chromosome number and in distribution. That aneuploid or dysploid numbers are brought about by the agency

of hybridism is a very important and significant fact to consider in connection with the explanation of the origin of aneuploidy in other plants, for the roses are not an exception in this regard.

The presence of 9 chromosomes in some cells of *Eleocharis palustris* recalls the behavior described by ROSENBERG (**27**) in *Crepis*, and he has used the occurrence of irregularities in chromosome distribution to explain the increase in chromosome number of recently originated *Crepis* forms. He believes that the *Crepis* species with three chromosomes have arisen from four chromosome forms by an occasional failing of two chromosomes in the pollen mother cells and embryo sac mother cell to pair in diakinesis, with the result that there are three paired and two unpaired chromosomes. When the chromosome distribution is irregular, some daughter nuclei receive five and others receive three chromosomes. If like gametes met like, the result would be the origin of three and five chromosome species of *Crepis*.

The work of WINGE (**29**) on crossing as the cause of polyploidy has been well established. In *Rubus* and *Crataegus*, LONGLEY (**19, 20**) has investigated the causes of polymorphy, and has come to the conclusion that the species have been multiplied by hybridization. In *Crataegus* he finds three classes: (1) diploid species in which pollen formation is normal; (2) triploid and tetraploid species that show irregularities in their chromosome distribution and are accompanied by polycary and polyspory; and (3) triploid and tetraploid species that are unable to form pollen grains and are sterile because they are the products of distant crosses. In *Rubus* he finds two classes: (1) diploid species in which meiosis and pollen formation are normal; and (2) polyploid species which are triploid, tetraploid, pentaploid, and octoploid. These polyploid forms are seemingly hybrid because characterized by irregularities in chromosome distribution which leads to polycary and polyspory. The fact that species have been modified by hybridization in their natural habitats is of fundamental importance in the consideration of other groups.

HOLMGREN (**15**) investigated *Erigeron micranthus*. Usually there are 13 pairs of chromosomes; frequently there are only 11 or 12, and the univalents which are formed as a result of the weakened affinity behave as supernumeraries and are distributed in varying

ways to the daughter nuclei. This is an indication that this species was derived from parents which differed in their chromosome constitution but very little. TISCHLER (31) regards these conditions in *Crepis* (reported by ROSENBERG) as being due to a like cause.

The Boston fern has been the subject of much study regarding its variations, which have been regarded as mutations. This fern is marked by its sterility and its great vegetative vigor. The apparent cause of polymorphism in this species has been described by JEFFREY and ROSCOE (18). The reduction divisions are quite abnormal, and there are conditions of lagging univalent chromosomes such as are found in known hybrids. Sporangia are shrunken and the development of the spores is generally entirely abortive. Accordingly this so-called mutant fern is to be regarded as a hybrid. Hybrid species of ferns with a high degree of probability often originate in greenhouses as well as in nature.

Many of the species regarded as examples of mutation have been shown to be of heterozygous origin. More recently *Drosophila melanogaster* has been shown to be abnormal in its divisions, both as to the presence of more than the expected number of chromosomes, and as to the absence of an equatorial plate typical in the metaphase, and the extrusion of chromosomes into the cytoplasm. These peculiarities may be considered to indicate the heterozygous origin of this much investigated insect.

The striking sets of homologous chromosomes described here in the small sedge *Eleocharis acicularis* have previously been reported in both plants and animals.

OSAWA (22) found trivalents occurring in triploid races of *Morus*, and by hybridizing several diploid species and races he was able to duplicate the trivalent condition. BELLING (1) has investigated triploid races of *Canna*. The chromosomes formed triads, and in the heterotypic split, and passed, two to one pole and one to the other. About half the pollen grains were devoid of cytoplasm. In regard to this condition in *Canna*, TISCHLER (31) states "zweifellos als Bastarde."

The recent investigations of maize have revealed the presence of trivalent chromosomes. LONGLEY (21) reports them in a cross between *Zea mays* and *Euchlaena perennis*. RANDOLPH and Mc-

CLINTOCK (23) still more recently have reported trivalents in triploid races of *Zea mays* which apparently were crosses between a dilute sun red race and a heterozygous tunicate tassel.

The question as to the order of succession of the reduction and the equation divisions has been the subject of considerable discussion. WILSON (34) states:

> This long disputed question must obviously rest upon our means of identification of the reduction divisions and . . . . such identification can only be made with complete certainty in cases where the synaptic mates are visible, distinguished by differences of form, size, structure, or mode of attachment.

A comparison of the polar views of the heterotypic metaphase plates, together with profile views of the same and the conditions found in the corresponding phases of the homeotypic, apparently leads to the conclusion that the reduction and the equation divisions are mixed in *Eleocharis acicularis*. The case of *E. palustris* is rather remarkable because there has not only been a doubling of the number of chromosomes, but also the addition of odd units. The extra individuals may possibly be explained by the evidence of non-disjunction, or by the weakened affinity seen in the few cases in the material from Spot Pond and Chestnut Hill, where 9 chromosomes were found in the metaphase plates. The material used in this investigation, however, may not be entirely representative of the species. As the writer has studied material from only seven stations, the exact method of duplication may reveal itself in further investigations.

A notable instance of a tetraploid form arising from diploid stock is found in the case of *Primula kewensis* (8). This plant arose as a hybrid in 1899 between *Primula floribunda* and *P. verticillata* at Kew Gardens. A peculiarity analogous to the quadrivalents seen in the large forms of *Eleocharis palustris* was found in the temporary linking of two bivalent chromosomes in the heterotypic division, which association persisted until the beginning of the anaphase. Like conditions have been reported in *Rosa wilsoni* by BLACKBURN and HARRISON (3). The parents of this species are *R. pimpinellifolia* and some *Tomentosa microgene*. CLAUSEN and GOODSPEED (7) found tetraploid forms in *Nicotiana* hybrids resulting from a cross of *N. glutinosa* (12×) × *N. tabacum* (24×). They claim it to be an experi-

mental verification of WINGE's hypothesis that higher chromosome numbers are derived from lower ones by crossing. TSCHERMAK and BLEIER (32), in their account of *Aegilops* and *Triticum* hybrids, believe also with WINGE that polyploidy has come about through crossing. The species *T. ovata*, *T. dicoccoides*, and *T. durum* possess 14 x chromosomes, but in fertile crosses there is a doubling and consequently there are 28.

Similar conditions have been reported in *Digitalis* crosses by HASSE-BESSELL (9), and in sugar cane by BREMER (5).

Conclusions

The cytological conditions described in the genera under discussion are remarkably correlated to the taxonomic variability recorded for the different species. In *Eleocharis* it has been shown how higher numbers have probably arisen and also how they have become modified in *E. palustris*. In this species in the stouter and taller races the number of chromosomes has become doubled and been further modified by some means; perhaps by non-disjunction of the chromosomes. This doubling has most probably come about through hybridization, and as a consequence further modifications have arisen.

The plants of *Eleocharis acicularis* investigated also present conditions that may be interpreted as indicating hybrid derivation. The peculiarities in the union of homologous chromosomes, unstable conditions in pairing, and lagging of chromosomes seem, in the light of known conditions of the origin of such structures, to mark the heterozygous nature of this plant. The weak pairing of the chromosomes seen in *E. obtusa* resulting in irregularity, together with the known variability of this plant, indicate that it may have suffered hybrid contamination.

These conditions, in a genus that is known upon the whole to be comparatively stable taxonomically, provide significant indication of the mode of origin of aneuploidy in other groups, namely, through crossing. *Cyperus* further presents evidence that falls in line with other genera investigated. This is illustrated by *Cyperus filiculmis* var. *macilentus* and also probably by the variety of *C. strigosus* here described. In view of the cytological evidence presented, it is to be concluded that *Eleocharis* and *Cyperus* are being modified by cross-

ing, and it is highly probable that the same situation has also prevailed in the past.

An interesting question arises in regard to the conclusions reached by HEILBORN in *Carex*. He came to the conclusion that the aneuploidy found in this genus is the result of mutation and has no obvious connection with previous hybridism. It seems unlikely, in view of the conditions here recorded, that this point of view can be maintained, at any rate for the genera studied in this investigation.

Summary

1. *Eleocharis*, so far as studied, has the following aneuploid chromosome numbers: 5, 8, 8–9, 15, 18, 19, and 26–29.

2. *Cyperus*, so far as studied, has the following aneuploid chromosome numbers: 17, 21, 48, 54, 73, and variable.

3. *Dulichium arundinaceum* has 15 chromosomes.

4. *Eriophorum virginicum* has 29 chromosomes.

5. Conditions similar to those found in known hybrids have been discovered in *Eleocharis* and *Cyperus*.

6. Cytological indication of hybrid origin is clearly correlated with taxonomic variability and polymorphy.

7. Hybridism is offered as a probable explanation of the aneuploidy found in the Cyperaceae.

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EXPLANATION OF PLATES VIII, IX

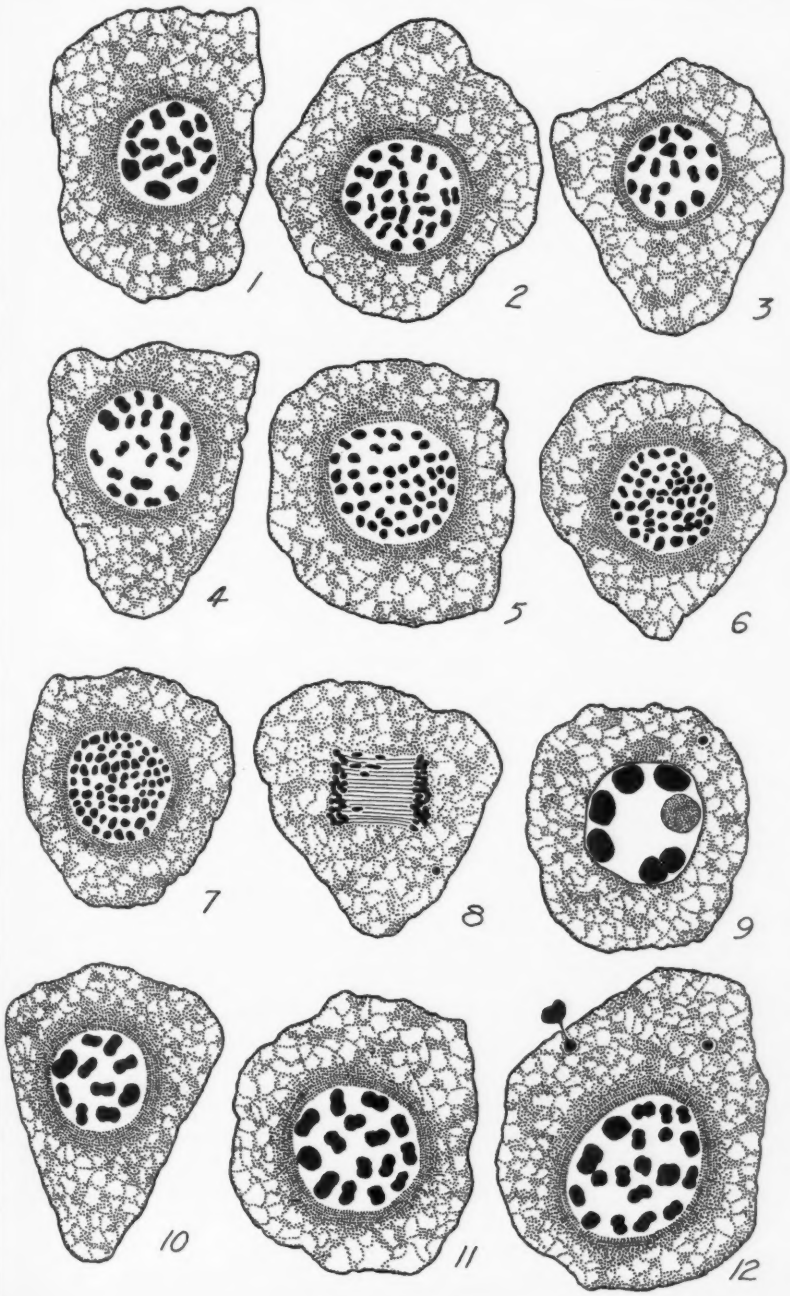
PLATE VIII

FIG. 1.—*Dulichium arundinaceum*, heterotypic metaphase.
FIG. 2.—*Eriophorum virginicum*, heterotypic metaphase.
FIG. 3.—*Cyperus dentatus*, heterotypic metaphase.
FIG. 4.—*Cyperus* sp. (New Zealand), heterotypic metaphase.
FIG. 5.—*C. erythrorhizos*, heterotypic metaphase.
FIG. 6.—*C. esculentus*, heterotypic metaphase.
FIG. 7.—*C. filiculmis* var. *macilentus*, heterotypic metaphase.
FIG. 8.—Same, heterotypic anaphase.
FIG. 9.—*Eleocharis obtusa*, diakinesis.
FIG. 10.—*Eleocharis* sp. (New Zealand), heterotypic metaphase.
FIG. 11.—*E. tuberculosa*, heterotypic metaphase.
FIG. 12.—*E. capitata*, heterotypic metaphase.

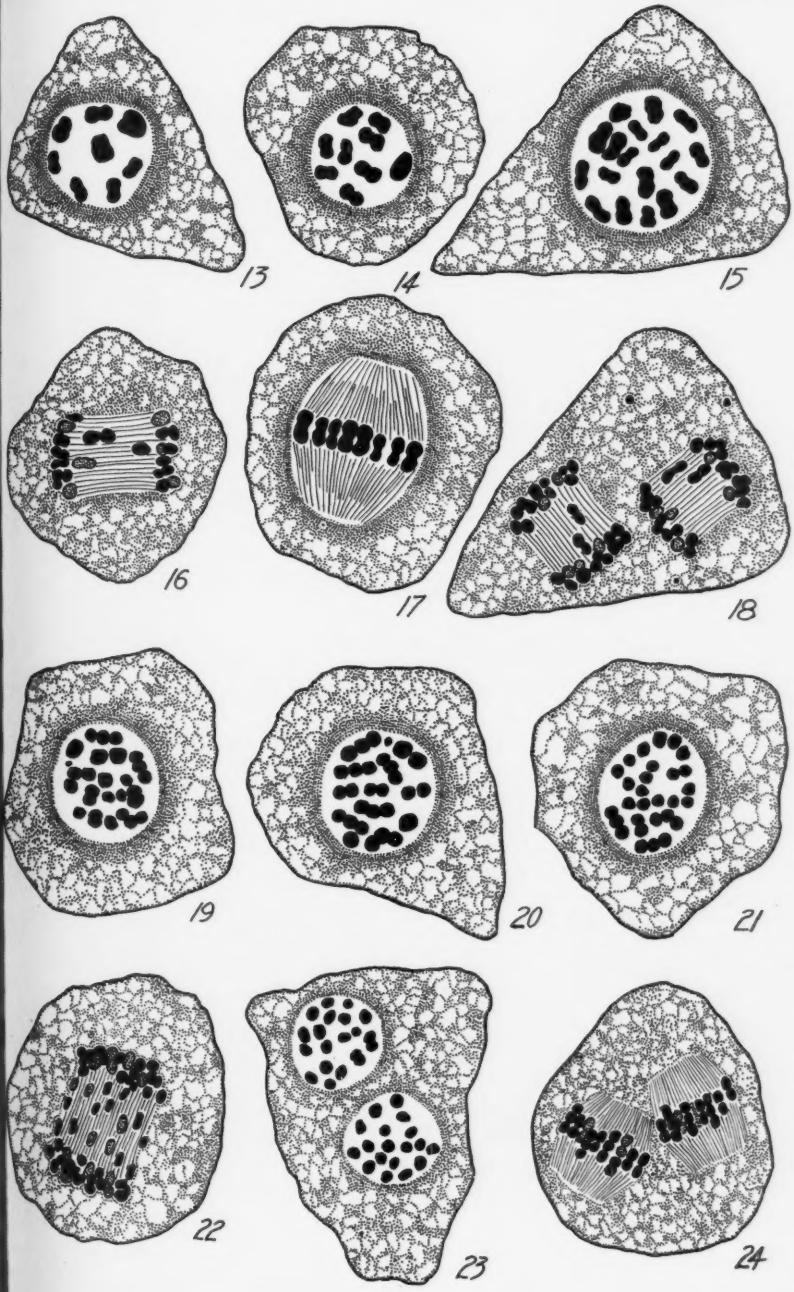
PLATE IX

Eleocharis palustris

FIG. 13.—Heterotypic metaphase showing 8 chromosomes.
FIG. 14.—Heterotypic metaphase showing 9 chromosomes.



HICKS on CYPERACEAE



HICKS on CYPERACEAE

FIG. 15.—Taller and stouter forms, showing 18 chromosomes.

FIG. 16.—Heterotypic anaphase.

FIG. 17.—Taller and stouter forms, showing side view of heterotypic metaphase.

FIG. 18.—Homeotypic anaphase.

Eleocharis acicularis

FIG. 19.—Heterotypic metaphase showing 25 chromosomes.

FIG. 20.—Twenty-nine chromosomes.

FIG. 21.—Twenty-nine chromosomes.

FIG. 22.—Heterotypic anaphase.

FIG. 23.—Homeotypic metaphase, polar view.

FIG. 24.—Homeotypic metaphase, side view.

MORPHOLOGY OF SPOROPHYTE OF MARCHANTIA DOMINGENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 394

EMMA N. ANDERSEN

(WITH THIRTY-FOUR FIGURES)

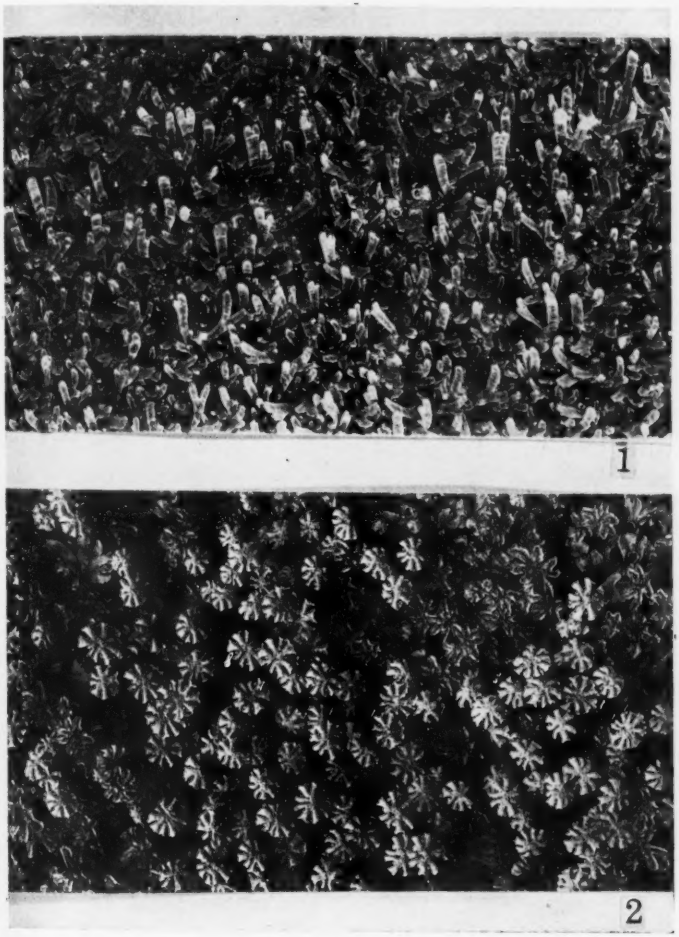
In reviewing the literature on *Marchantia*, many studies, discussions, and references dealing with *M. polymorpha* were found. As early as 1874 KIENITZ-GERLOFF (**15**) contributed a detailed study of it. About five years later, LEITGEB (**18**) made a study of *M. chenopoda* and *M. polymorpha*. CAVERS (**6**) contributed to another phase of *M. polymorpha*. KÜTZING (**16**) made a study of the elaters of *Marchantia* very early indeed. Finally, DURAND (**8**) speaks of *M. polymorpha* as being the most accessible as well as the most easily studied species of the genus, and feeling that it was commonly used in the laboratories, he deemed it desirable to give a somewhat complete series of illustrations and descriptions. While *M. polymorpha* has received considerable attention, *M. domingensis* has received very little. Aside from the work of EVANS (**9**), only one other treatise was found, which dealt with the origin of the gametophore, by LUCILE CAPT (unpublished).

It is undoubtedly true, as CAVERS (**6**) states, that the sporogonium affords little guidance to the phylogeny of the Marchantiales, except in a general way; nevertheless (as he also admits), taken with other characters it is of value and must receive careful study. Having available an abundance of *Marchantia domingensis* material, it was suggested by Dr. W. J. G. LAND that a study of the sporophyte be made.

Material and method

Marchantia domingensis Lehm. and Lindenb. was grown in the University greenhouse at Lincoln, Nebraska. The room was kept at approximately 22° C. and the cultures were watered nearly every day. Some of the flats were kept for several weeks under glass until

near time of fruiting, when the glass was removed. The cultures grown under glass became morphologically modified. The thalli were conspicuously elongated (fig. 1), and fruiting was initiated a little



FIGS. 1, 2.—Fig. 1, gametophytes grown under glass; fig. 2, gametophytes showing cupules, archegonial branches, and old antheridial branches; ×1.

earlier than in the cultures grown in open flats; however, abundant fruit was produced in both cases (fig. 2). A few flats were given additional light by using a 100 watt mazda lamp, thus giving approximately 6 hours of artificial light in addition to the normal daylight. Cultures receiving the additional light (starting on November 22) were beginning to produce fruit on December 18, 1925. The thalli of the cultures not receiving additional light had not fruited by that time, but surpassed them in both size and color of thallus. The response of *M. domingensis* to the "long day" agrees with what WANN (24) found in *M. polymorpha* in 1922. Since the fruits from the thalli receiving only normal daylight appeared more vigorous than the plants securing additional light, they were selected almost exclusively in this study. The greater number of the figures were secured from collections made the third and fourth weeks of April, 1927. The collections were made in the forenoon and afternoon, as well as at one and three in the morning.

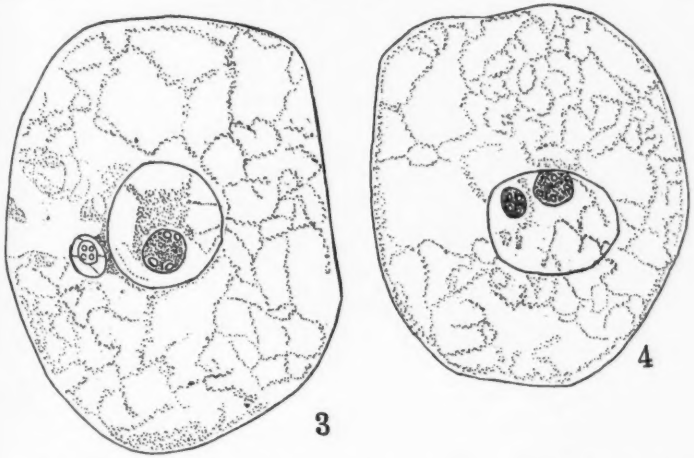
Material was fixed in the following solutions: acetic alcohol, Carnoy's, chromo-acetic, corrosive sublimate, Flemming's, formalin alcohol, platinum chloride, and formalin acetic alcohol. The formalin acetic alcohol and hot corrosive sublimate gave the most satisfactory results. A great number of stains were also employed, but the iron-alum haematoxylin method was for the most part used. Material was cut 5–12 μ in thickness.

FERTILIZATION

Fertilization has been reported by a number of workers in various liverworts. A remarkable similarity in the relative size of egg and sperm exists in the contact stage. GARBER (10) on *Ricciocarpus*, BLACK (2) on *Riccia*, MEYER (19) on *Corsinia*, DUPLER (7) and WOODBURN (25) on *Reboulia*, GRAHAM (11) and HAUPT (13) on *Preissia*, all show the egg nucleus about twice the diameter of that of the sperm. In *Marchantia domingensis* the egg nucleus was about three times the diameter of that of the sperm.

About thirty cases of fertilization were observed in *Marchantia*. In the first place, when the sperm has not yet come into contact with the egg nucleus, a denser mass of cytoplasm paves the space between it and the egg nucleus. This condition HAUPT (13) also

illustrates in the case of *Preissia*. The egg and sperm nucleoli are very conspicuous, each being vacuolate. The egg nucleolus usually has a dense mass of chromatin-like material surrounding it, with more on one side than the other. This mass may be surrounded by another less dense material, or granular threads may protrude from it (fig. 3). Some egg nucleoli were surrounded by reticulate material (fig. 4). The sperm nucleus, especially when found outside the egg



FIGS. 3, 4.—Fig. 3, egg with male nucleus not yet in contact with female; fig. 4, male nucleus within female nucleus; ×1000.

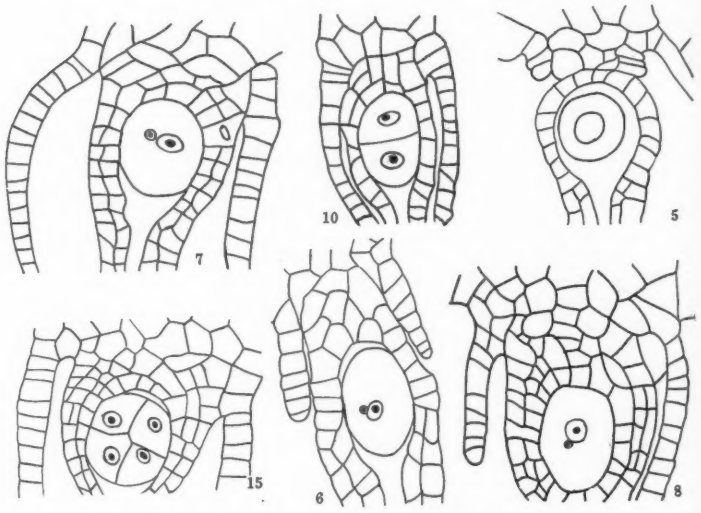
nucleus, usually had the chromatin-like material surrounding the nucleolus, but also had a few strands projecting from it (fig. 3). Centrosomes and astral rays were not seen.

EMBRYO

In the very early stage, the fertilized egg is surrounded by a venter which consists of a single layer of cells. The base of the venter and the cells beneath, composed of three or four cells in width, will be designated basal cells. The cells beneath the venter give rise to a cylindrical structure, a pseudoperianth, one layer in thickness and a few cells in height (fig. 5). A little later it is noticeable that the basal tissue, the pseudoperianth, and the venter have

been stimulated to enlargement or cell division (fig. 6). The basal tissue becomes more massive, the pseudoperianth increases in length, and there occur periclinal and radial divisions in the venter, usually two successive periclinal divisions, resulting in a 3-celled structure, the calyptra (figs. 7, 8).

The first division of the fertilized egg gives rise to a transverse

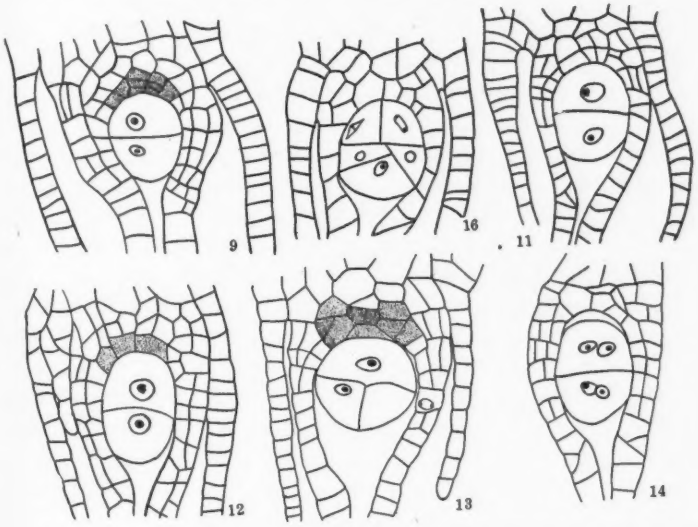


FIGS. 5–8, 10, 15.—Fig. 5, early stage of fertilized egg; fig. 6, later stage of fertilization; figs. 7, 8, development of surrounding structures; fig. 10, first division of fertilized egg; fig. 15, octant stage of sporophyte; ×300.

wall, which may vary in the angle that it makes with a plane which is perpendicular to the major axis of the archegonium. The time of formation of the first wall varies. In some cases it is not formed until extensive activation has taken place in all three regions, namely, the basal tissue, pseudoperianth, and venter (fig. 9); in others it may be found when the basal cells and to a greater extent the pseudoperianth have undergone several divisions, while the venter alone has remained more or less dormant (fig. 10); in still others it may occur when cell division has taken place in all three regions, relatively less, however, than was found in the first situation mentioned

(fig. 11). The first row of basal cells is frequently filled at this stage with granular material (fig. 12). Very soon another row of the basal tissue may also become very granular (fig. 9).

In *Corsinia*, MEYER (**19**) figures the venter three to four cells thick at the time of fertilization; later, when the sporogenous tissue has been differentiated, he finds it to be in places six cells thick, with



FIGS. 9, 11–14, 16.—Figs. 9–12, variations in surrounding structures when first wall is formed; fig. 13, division of epibasal cell; fig. 14, division of both cells; fig. 16, anticlinal walls in epibasal region; ×300.

a massive neck as well. LANG (**17**) found in *Cyathodium foetidissimum* that with the first division of the embryo the venter of the archegonium becomes two cells thick; and when the sporogenous tissue is clearly defined by the dense and deeply staining contents of its cells, it becomes three or four cells thick.

CAMPBELL (**4**), working with *Targionia*, states that with the first divisions in the embryo, a series of periclinal walls occurs in the venter, making it two cells thick; later it undergoes further divisions, forming a calyptra four or more cells in thickness. MISS STARR (**22**) finds in *Aytonia* that all the cells of the venter have divided when

the first division of the embryo occurs, and that growth continues in the basal tissue, the venter, and the lower part of the neck until a large amount of tissue is developed about the embryo. In DUPLER's (7) discussion of the calyptra and involucre in *Reboulia*, he states that the former grows apace as the embryo develops, ultimately forming a several-layered and relatively somewhat massive structure. CAVERS (5) finds in *Fegatella* that fertilization is immediately followed by the appearance of tangential walls in the cells of the venter, the calyptra finally becoming five or six cells thick. BOLLETER (3), studying *Fegatella*, shows that even before the first transverse wall has appeared the calyptra has attained a wall three cells in thickness. DURAND (8), in his work on *Marchantia polymorpha*, found that while development was proceeding in the embryo, periclinal divisions began also in the walls of the venter and continued until two or three layers of cells were formed at the sides of and above the embryo.

In ascertaining the prevailing condition of the surrounding tissues in early embryogeny, it may enable us to determine the cause for the diversion of tissue in the various regions. *Marchantia domingensis* is found in some cases to differ from the related forms that have been reported, relative to the calyptra. It may pass through fertilization, form the first transverse wall, and may even attain the octant stage while the venter is still one-layered. That this factor alone may alter its subsequent development seems plausible.

After the first division of the fertilized egg, the epibasal cell divides in a plane perpendicular to the first wall laid down (fig. 13). Exceptions occur; the hypobasal may divide first, or the two may divide simultaneously, forming the quadrant (fig. 14). Following the formation of the quadrant, there occurs a vertical division of each cell, which cuts at right angles to the other two faces, resulting in the octant stage (fig. 15). This stage does not occur when the venter, basal cells, or pseudoperianth have made a definite amount of progress, but, as in the case of the formation of the first wall, its appearance is variable.

HAUPT (13), reviewing the literature on the early embryogeny of liverwort sporophytes, distinguishes two types of embryos, the filamentous and what may be called the octant type. Under the

octant type appears the work on *Riccia* by KIENITZ-GERLOFF (14); on *Riccia*, *Targionia*, and *Hypernantron* by CAMPBELL (4); on *Corsinia* by MEYER (19); on *Cyathodium foetidissimum* by LANG (17); on *Cryptomitrium* by ABRAMS (1); and on *Marchantia polymorpha* by DURAND (8). To this list may be added the work of HAUPT (13) on *Preissia*.

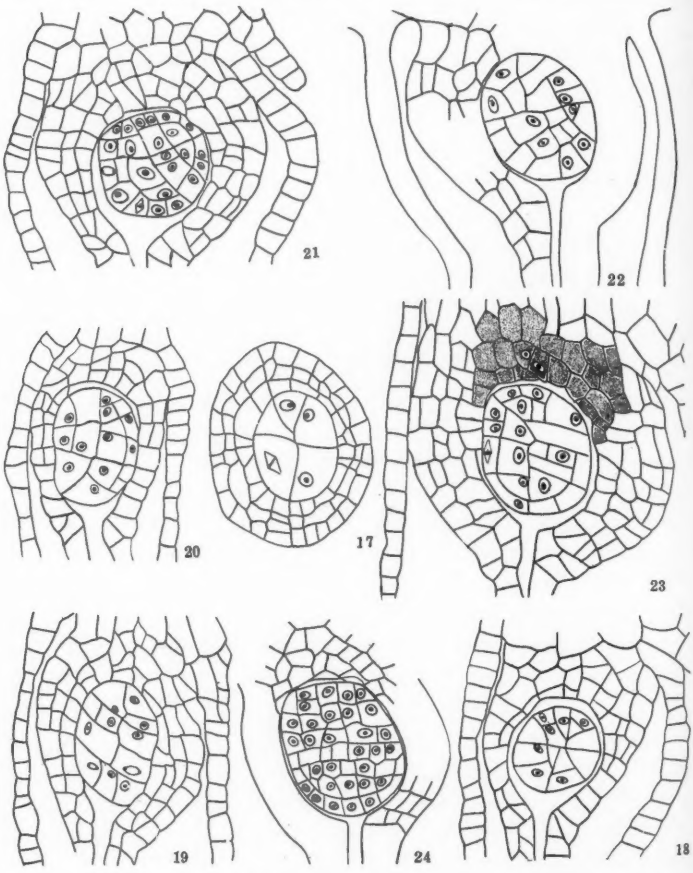
That species in the same genus do not necessarily show the same type of early development is demonstrated by the two species of *Cyathodium*. *C. cavernarum* by LANG (17) illustrates the filamentous type, while *C. foetidissimum* illustrates the octant type. *Targionia* by O'KEEFE (21), *Fegatella* by BOLLETER (3), and *Dumortiera* by CAMPBELL (4) have been found to belong to the filamentous type.

Anticlinal walls make their appearance very soon after the octant stage has been formed (figs. 16, 17). The anticlinal divisions are followed by periclinal and radial walls (figs. 18, 19). Repeated cell divisions result in the formation of a somewhat globular mass. After the first anticlinal walls are formed in the octant stage, no definite procedure could be ascertained in the successive cell divisions. In studying median longitudinal sections, while the outstanding character is the large amount of irregularity displayed, there is a decided tendency toward the formation of horizontal rows in the epibasal and vertical rows in the hypobasal portion (fig. 20). The significance of this manner of cell division is obvious, especially the procedure in the hypobasal region, when subsequently it becomes very clear that it is the conducting region and much nourishment is to be transported through it. Even when the first division of the embryo forms the first wall, the basal cells full of granular material which takes a deep stain would indicate a storage region which is to be drawn upon by the developing embryo. Fig. 21 shows the greater development to have taken place in the epibasal region; on the other hand, fig. 20 shows the hypobasal region to be more developed.

Divisions in the two upper or two lower octants do not proceed at the same rate, nor are they duplicates of each other (figs. 20–22). The calyptra divides again and becomes four cells thick.

When an outer layer of cells has been definitely cut off by periclinal divisions, it becomes the sporangium wall. In tracing the development of the epibasal region, early transverse rows of cells

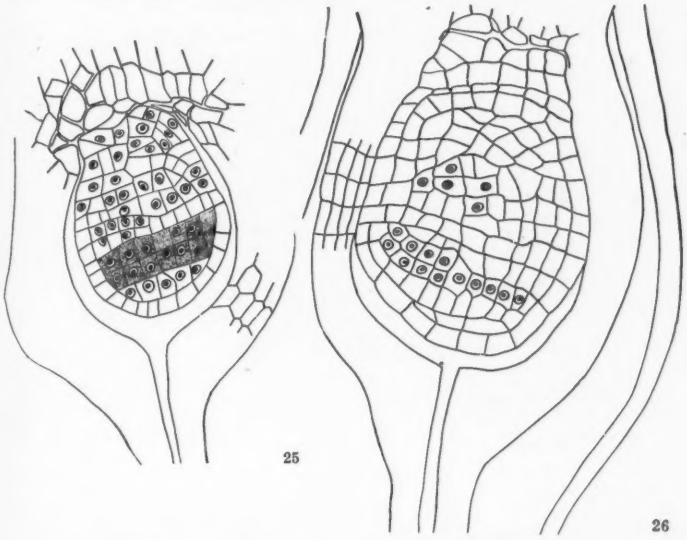
are found within the sporangium wall (fig. 22). At a later stage three rows of cells are found within the sporangium wall, the third row



FIGS. 17–24.—Fig. 17, anticlinal division in hypobasal region; figs. 18, 19, periclinal divisions in both hypobasal and epibasal regions; fig. 20, hypobasal region elongated; figs. 21, 22, stages in development of both upper and lower regions; figs. 23, 24, three rows of cells in epibasal region within sporangium wall; ×300.

having arisen by vertical as well as by transverse divisions occurring in the second row (figs. 23, 24). Radial divisions have taken place in

the sporangium wall also. The second row of cells appears to divide and gives rise to the sporogenous tissue (figs. 25, 26). The first row beneath the sporangium wall (fig. 25) divides, producing a sterile cap or disk two cells in thickness (fig. 28). That no further division takes place in this sterile mass or cap is proved by examination of the following stages. This sterile apical cap is of common occurrence



FIGS. 25, 26.—Two rows of sporogenous tissue formed from second row of cells in epibasal region; ×300.

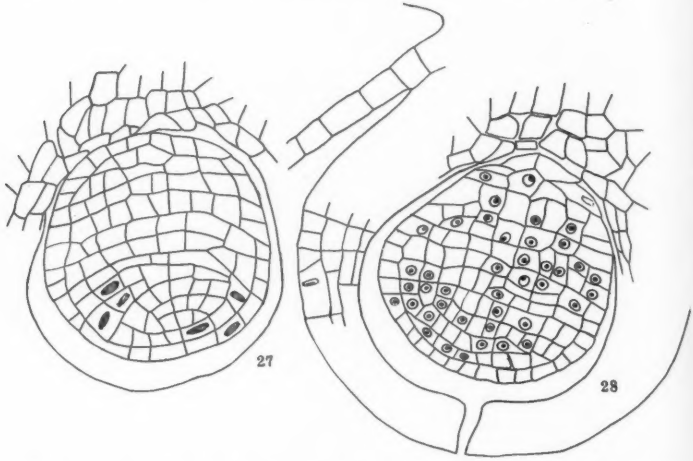
in all the upper genera of the Marchantiales, and also in the intermediate genus *Cyathodium*.

At the time that the sporogenous tissue is being differentiated in the epibasal region, many more cell divisions have apparently occurred in the hypobasal region (figs. 24, 25). The foot has been definitely formed; the two rows of cells adjacent to the basal cells are enlarged and deeply stained, and have the appearance of basal tissue (fig. 26). The shape of the sporophyte has been modified (fig. 25). The distal end where vertical divisions are more numerous than transverse (fig. 27) tends to broaden that region, resulting in the sporophyte presenting a *Lycoperdon*-like structure.

LANG (17), in speaking of the two tiers of cells in *Cyathodium* forming an apical disk, states:

> the lowest tier can be traced back to the upper portion of the sporogenous tissues. A single layer of the latter at the upper limit of the group does not separate into spore mother cells, but remains as a continuous layer in contact with the uppermost sterile segment and forms a part of the apical disk.

In *Reboulia*, according to CAVERS (6), the apical portion of the capsule wall with its well developed cap falls away in fragments at



FIGS. 27, 28.—Fig. 27, vertical divisions occurring in epibasal region; fig. 28, sterile cap two cells in thickness; ×300.

the time of dehiscence. ABRAMS found in *Cryptomitrium* that the capsular wall at the apex consisted of two rows of cells. CAMPBELL found in *Hypernantron* that sometimes a cap of sterile cells formed the apex of the sporogonium. CAVERS (5) states that the cap in *Fegatella* appears to be derived from the uppermost portion of the sporogenous tissue. When dehiscence occurs, a line of cleavage is formed around the upper portion of the capsule just outside the apical cap. This crack is irregular and wavy, but it corresponds in general with the junction between the apical cap and the rest of the capsule wall. Valves are formed in the capsule by longitudinal splitting, but the apical cap remains, either becoming loosened all around

and severed from the capsule or remaining attached to one of the valves. CAVERS (6) also finds that the apical disk not only in *Fegatella* but in *Lunularia* and *Dumortiera* is also thrown off as a lid; on the other hand, he states that in other genera the apical cap (composed of an imperfect or loose layer of cells) lies within the normally one-layered capsule wall, but the capsule dehisces by means of teeth extending to the apex. CAVERS also discusses the modified apical cap as illustrated in *Preissia commutata*, where it is a lens-shaped structure. The cells bear ringlike fibers and elater-like cells project from them.

In *Marchantia domingensis*, the two rows of sterile cells designated apical cap or disk, lying between the sporogenous layers and the sporangium wall, increase in size and resemble the granular basal tissue. They probably function as conducting tissue, since they are only one cell layer removed from the massive tissue of the archegonial neck on one side while they are in direct contact with the sporogenous layer on the other (fig. 29).

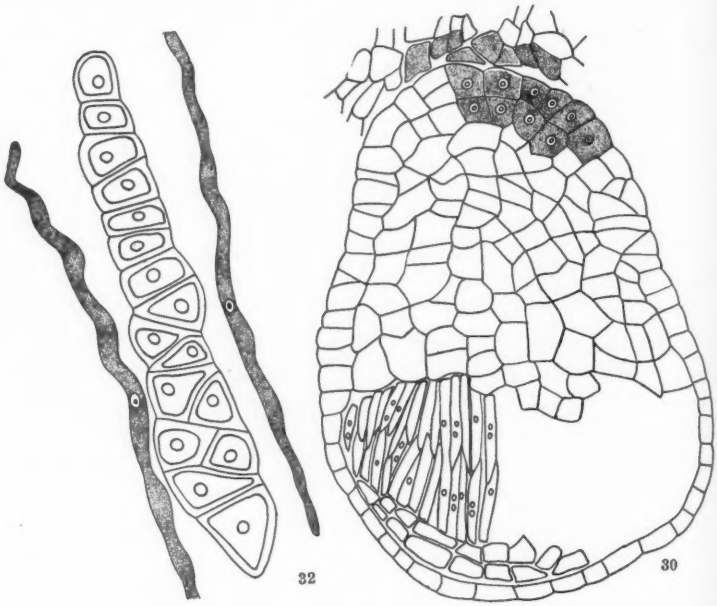
The third row of sterile cells from the sporangium wall, lying next to the first transverse wall of the fertilized egg, divides at least once in transverse section (figs. 25, 27). That it may be considered as additional tissue to the seta seems apparent. It has long been recognized by various writers that the first transverse wall divides the fertilized egg into an upper (epibasal) and a lower (hypobasal) cell. The amount of tissue in the epibasal region that has been diverted in the various genera has also received attention.

MEYER and LEITGEB both worked on *Corsinia*. They are of the opinion that the hypobasal cell contributes to the capsule in many cases at least. In *Reboulia*, HAUPT (12) found that the first transverse wall formed separates the cell which forms the foot from that which is to form the seta and the capsule. ABRAMS states that in *Cryptomitrium* usually the first transverse division is the line of demarcation between the capsule and the foot.

According to KIENITZ-GERLOFF (14), the foot and seta in *Preissia* are derived from the hypobasal cells, while the epibasal cell forms the capsule. CAVERS (5) finds in *Fegatella* that the lower (hypobasal) portion contributes only to the foot, while the stalk and capsule are both derived from the epibasal portion. In *Marchantia poly-*

morpha, DURAND (8) speaks of the first division wall as separating the stalk and capsular valves, and he believes this is usually the case; however, he states this is not always true.

It is noteworthy that while several genera in the upper stretches of the Marchantiales have been reported to have diverted epibasal tissue, none, so far as reported, have reduced the sporogenous layers to the extent that *M. domingensis* has.

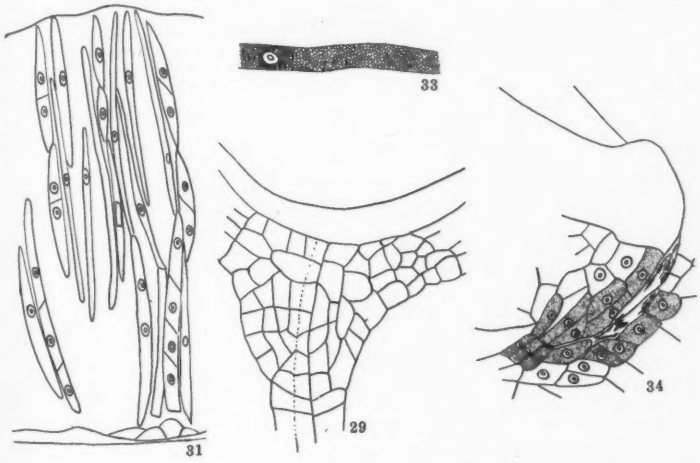


FIGS. 30, 32.—Fig. 30, sporogenous and non-sporogenous cells, ×300; fig. 32, spore mother cells and elaters; ×600.

When the sporogenous mass can be distinguished by its deeply staining cells, the two layers elongate greatly, pushing past each other to a considerable extent (fig. 30). It appeared that every other cell remained uninucleate while the alternate ones divided (fig. 30). In a stage slightly older another division had occurred, resulting in a row of four cells in each half of the sporogenous mass (fig. 31). After one more division, these cells may form very thick walls and become spore mother cells. They may divide again (fig. 32), how-

ever, making the fourth successive division since the time when practically half of it was diverted to form elaters. This situation differs from the condition in *M. polymorpha*. O'HANLON (**20**) states that the elaters of *Marchantia* are sister cells of sporogenous cells which undergo five divisions before the spore mother cells appear.

After the disappearance of the thick walls surrounding the spore mother cells, the usual tetrad formation is initiated. During the



FIGS. 29, 31, 33, 34.—Fig. 29, massive archegonial neck; fig. 31, second division of sporogenous cells and uninucleate elaters; fig. 33, elater containing peripheral layer of minute cytoplasmic vacuoles; ×600; fig. 34, half of longitudinal section of foot showing elongated lateral cells; figs. 31, 29, 34; ×300.

maturation of the spores the sporangium wall becomes strengthened by U-shaped thickenings. The elaters which at first are mucilaginous soon become granular, and remain in this condition up to the spore mother cell stage (fig. 30).

At the time of the disappearance of the spore mother cell wall, the elaters show a peripheral layer of minute cytoplasmic vacuoles (fig. 33). As elongation in the elater proceeds, larger vacuoles occur between bands of smaller ones. Later, in place of the small cytoplasmic vacuoles, spiral thickenings occur. This situation confirms the finding of STOVER (**23**), who states that pitted thickenings arise by the vacuolation of the cytoplasm, the thickening being laid down

by the thicker portion of the cytoplasm. He also believes that the type of tracheae varies with the amount of elongation.

Foot and seta

Since the basal tissue becomes granular when the first transverse wall of the embryo is formed, it indicates that this is the region which contributes the greatest amount of nutrition to the developing embryo (fig. 9). It is the region through which the nutrition is constantly moving. On the sides where the seta makes contact, and above where the sporangium wall may come in contact with the calyptra, it is intermittent, due to differential growth. Later the space between the seta and the calyptra increases. The lateral cells of the foot (fig. 34) which keep in contact with the gametophyte become greatly elongated, changing the shape of the basal portion. The tendency for the seta to elongate vertically and the cells of the foot to become greatly elongated gives the pileus-shaped foot. The lateral cells of the foot, which at first elongate, soon undergo transverse division. The cells of the seta, arranged in rows, connecting the much enlarged cells of the foot with the sporogenous mass, contain vertical walls which appear thicker than the transverse walls. The cells do not lose their nuclei. The two deeply staining rows of the foot of *M. domingensis* show a marked contrast to the illustration as given by Durand (**8**) in *M. polymorpha*, where all the cells of the foot appear to be deeply staining. At an early stage, particularly when the sporogenous tissue has undergone two divisions in *M. domingensis*, the seta is found to be much greater in length than it is in *M. polymorpha* at this stage. The cells of the former, constituting the seta, display greater linear arrangement than similar cells in the latter do. Whether these minute differences in the tissues are indicative of greater specialization may at least be questioned.

Summary

1. At the time of fertilization of *Marchantia domingensis* both egg and sperm nucleoli are very conspicuous. Chromatin-like material surrounds the nucleolus of the egg, while granular strands usually project from the sperm.

2. The time of the appearance of the first wall varies.

3. The early development of the sporophyte is the octant type which occurs in the greater number of the Marchantiae.

4. The epibasal cell gives rise to three regions, namely, an upper sterile region consisting of two rows of cells forming the apical cap; beneath the cap the two sporogenous layers; and underneath them another sterile region contributing to the seta.

5. The hypobasal cell forms the remainder of the seta and foot.

6. The apical cap functions as a conductive system between the massive neck and the sporogenous tissue.

7. The foot early differentiates two rows of deeply staining cells that retain this condition even after the tetrad formation.

8. The vertical walls of the seta appear thicker than most of the transverse walls, although they do not lose their nuclei.

9. The venter may not begin tangential divisions until after the octant stage has been formed.

10. Sporogenous cells undergo three or four divisions before spore mother cells are formed.

11. Sporogenous tissue consists of two transverse rows, practically half of which become sterile forming elaters.

12. Vacuolation of the peripheral cytoplasm in the granular elaters is responsible for the spiral thickenings occurring later.

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MORPHOLOGY OF NORTH AMERICAN SPECIES OF POLYGALA

THEO. HOLM

(WITH FORTY-TWO FIGURES)

Introduction

The absence of internal secretory organs is, according to SOLEREDER (7), characteristic of the Polygalaceae, with exception of the lysigenous secretory ducts and oil cells of many South American species of *Polygala*, which have been mentioned by CHODAT (1, 2), and which SOLEREDER recommends to be reexamined as well as the occurrence of peculiar granular masses resembling aggregated crystals, which VESQUE (8) found in the leaves of certain Brazilian species of *Polygala*. The discovery of these peculiar crystal-like bodies in the Brazilian species was compared by VESQUE with the epidermal secretions in violets from Chile, and he reached the conclusion:

Si nous considérons ces faits au point de vue de l' anatomie comparée, nous voyons que ce genre (*Polygala*) se comporte comme les *Viola* dont certaines espèces géographiquement limitées se distinguent également par une sécrétion particulière.

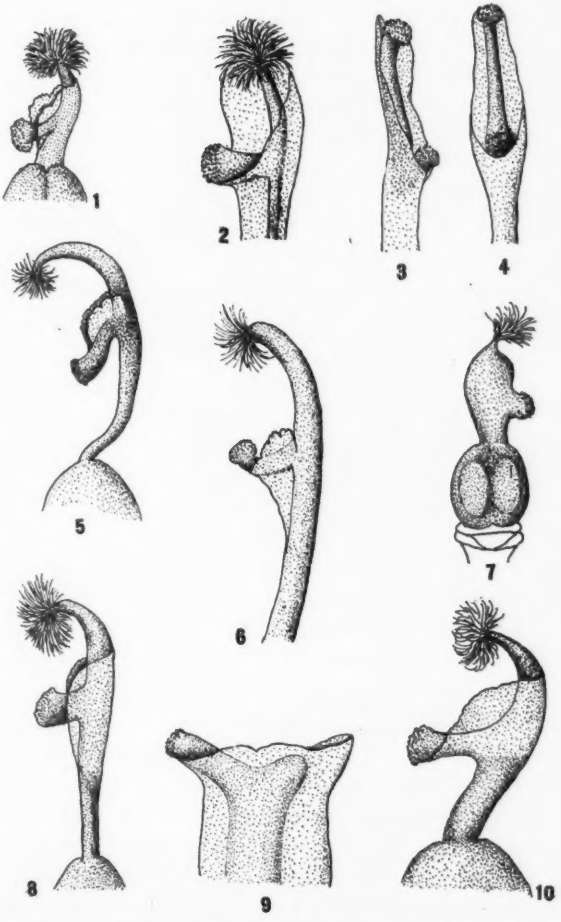
With regard to the Chilean violets, the discovery made by VESQUE was corroborated by REICHE (5), who had considerable material at his disposal, but so far as concerns the lysigenous ducts mentioned by CHODAT in *Polygala*, these have not been observed in other species. An anatomical study of *Viola* and *Polygala*, as these genera are represented in Maryland and Virginia, has shown that several species of the former show a structure corresponding with that of the Chilean species, so far as concerns the secretions, and that several species of *Polygala* do possess lysigenous ducts in leaves as well as in stems.

While studying *Polygala*, however, it was noticed also that the floral structure is quite characteristic in certain species, although not described in any very precise manner in the various botanical manuals. For instance, the structure of the style and stigmata is difficult to describe without figures, therefore some of the types represented

in my material are illustrated here. I have examined the following species of the section Orthopolygala Chodat: *P. polygama* Walt., *P. senega* L., *P. incarnata* L., *P. ambigua* Nutt., *P. curtissii* Gray, *P. mariana* Mill., *P. nuttallii* Torr. & Gr., *P. sanguinea* L., and *P. lutea* L.

Style and stigmata

The apex of the style is bicleft, and generally very unequally so. The posterior branch, the shorter one, has a typical, papillose, viscid stigma; while the anterior is terminated by a tuft of long, bifurcate hairs, but with no stigma. A very thin membrane is very conspicuous; in *Polygala lutea* (fig. 5) this covers only the upper face of the stigmatic lobe, while in *P. polygama* (fig. 10) it extends around the basal part of both style branches. In *P. incarnata* the membrane is confined to the posterior face of the style, extending from the stigmatic lobe a short distance beneath this. In *P. curtissii* (fig. 8) the membrane covers the base of both style branches, extending a short distance downward, and this structure recurs in *P. mariana* and *P. nuttallii*. In *P. ambigua* the relatively short style is surrounded by the membrane, which extends upward to near the apex of both style branches, a structure which recurs in *P. alba* Nutt. and *P. paniculata* L. In *P. senega* (fig. 7), on the other hand, there is no membrane, but otherwise the structure resembles that of the other American species. In *P. longicaulis* H.B.K. (fig. 2), collected in Porto Rico, the membrane is very long, extending above the apex with the hairy appendage, and a similar development of the membrane recurs in *P. tenuifolia* Willd. (figs. 3, 4) from Nerczynsk, Dahuria, where the anterior style branch is terminated by a globose, papillose, but not viscid stigma, while no tuft of hairs is developed. Finally, in *P. paucifolia* Willd. of the section Chamaebuxus DC. (fig. 9) the membrane forms a tube almost half as long as the whole style, and only the viscid stigma is visible, the barren branch of the style being very short and hidden within the membrane. So far as concerns the North American species, *P. paucifolia* and *P. senega* may be readily distinguished from the others; but with reference to those with membrane and a tuft of hairs, the difference depends only on the varied development of the membrane: small in *P. lutea* and *P. incarnata*, larger in the others. The difference in the

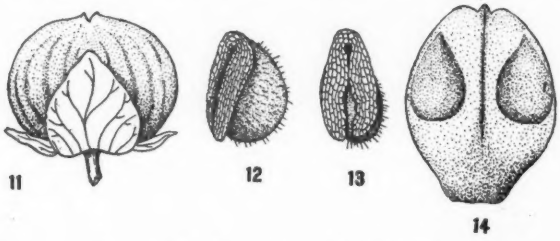


FIGS. 1–10.—Fig. 1, *Polygala ambigua*, upper part of ovary with style and stigmata; fig. 2, *P. longicaulis*, upper part of style with stigmata; fig. 3, *P. tenuifolia*, upper part of style with stigmata, side view; fig. 4, same, upper part of style with stigmata, front view; fig. 5, *P. lutea*, upper part of ovary with style and stigmata; fig. 6, *P. incarnata*, upper part of style with stigmata; fig. 7, *P. senega*, ovary with style and stigmata; fig. 8, *P. curtissii*, upper part of ovary with style and stigmata; fig. 9, *P. paucifolia*, upper part of style with stigmata; fig. 10, *P. polygama*, upper part of ovary with style and stigmata; ×30.

style and stigma structure is much more pronounced in the South American species, when we compare the numerous structures figured by CHODAT. In this respect the southern members of *Polygala* agree with the Andine violets, of which REICHE has figured a number of very singular structures exhibited by the style and stigma.

FRUIT

The fruit is a compressed, 2-celled capsule, wingless in the species enumerated; seeds are solitary in the cells, pendulous, more or less hairy, and conspicuously carunculate. Among the types of fruit and seed characteristic of some of the species may be mentioned: the thickish capsule, much broader than long in *P. senega* (fig. 11), and



FIGS. 11–14.—Fig. 11, *Polygala senega*, capsule with sepals, side view; ×14; figs. 12, 13, same, seeds, side and front view; ×20; fig. 14, *P. sanguinea*, capsule, side view; ×14.

the caruncle as long as the seed itself (figs. 12, 13); the large, thin-walled capsule of *P. sanguinea* (fig. 14) notched at the apex; the oblong capsule of *P. incarnata* (figs. 17, 18), and the caruncle about half as long as the seed itself (fig. 19); the small capsule of *P. ambigua* (figs. 23, 24), and the seed with its relatively short caruncle (fig. 25); the large, turgid capsule of *P. mariana* (fig. 29), and the distinctly apiculate seed with a short caruncle (figs. 30, 31); and the oblong capsule of *P. lutea* (fig. 34), notched at the apex, and the seeds with a long caruncle (figs. 36, 37). Characteristic of *P. lutea* also is the carpophore (fig. 35), distinctly 2-celled, which remains together with the wings, after the capsule has opened and the seeds dropped out; in all the other species examined the flowers fall off completely when the fruit has matured. The fruit and the seed thus show some specific differences, notably in *P. senega*, *P. sanguinea*, *P. mariana*, and

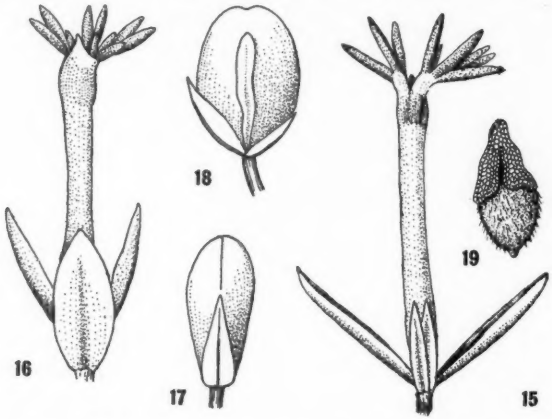
P. lutea; while the structure shown by *P. incarnata* and *P. ambigua* recurs in several of the other species of the section Orthopolygala.

Calyx and corolla

The flowers are strongly zygomorphic (the plane of symmetry being median); they have five free sepals, the two lateral ones of which are large, petaloid, and frequently project on each side like the "wings" of a pea flower; the other sepals are much smaller, sepaloid, and the odd one (dorsal) is generally a little longer than the anterior pair. There are five petals, of which the two lateral ones are either rudimentary or completely absent, as in our species, and the anterior (keel) is large, hollow, and carinate with the apex lobed or fimbriated, and more or less connate with the others. The shape, size, and color of the wings and petals show several distinct characters in the genus, even in the small number of species I have examined.

In *Polygala incarnata* (figs. 15, 16) the wings are relatively short, spatulate, and green with broad hyaline margins. The petals are united into a long slender tube, pale rose or purplish, and twice as long as the wings; the keel is crested at the apex in the manner of four bicleft, narrow lobes with two additional pairs, but much shorter, all spreading, and of a deep purplish color. In *P. senega* the small flowers are greenish white; the three small sepals are relatively broad and obtuse, and the wings are also broad, obtuse, and a little longer than the corolla. The petals are almost free, the two posterior being oblong, obtuse, and about as long as the broad keel with its crest of about ten short, obtuse lobes. In *P. ambigua* (figs. 20–22) the very broad, obtuse wings are a little shorter than the connate petals, of a white color with green midveins. The petals are pale greenish, and the small crest consists of one pair of short lobes. In *P. mariana* (figs. 26–28) the wings are relatively narrow, acuminate and unguiculate, purplish, one-nerved, and of about the same length as the corolla; the two posterior petals form a tube around the keel and are distinctly longer than this, including the pair of short, bifid lobes. While the tube of the two petals is pale yellow, the keel is hyaline with the crest deep yellow, but dark purplish when fading. The flower of *P. nuttallii* resembles that of the preceding species, but

the wings are of a paler purplish color. In *P. sanguinea* the wings are ovate-oblong, purplish above, whitish at the base, and the keel is white. In *P. lutea* the wings are 5-nerved, ovate-oblong, with a short, sharp, apical cusp, and longer than the petals; of these the two lateral form a tube, more or less emarginate at the apex (figs. 32, 33). The keel bears a crest of four bifid, short narrow lobes. The wings and the petals are of a beautiful orange color, and, as already stated, the wings persist for a long time after the fruit has matured and the



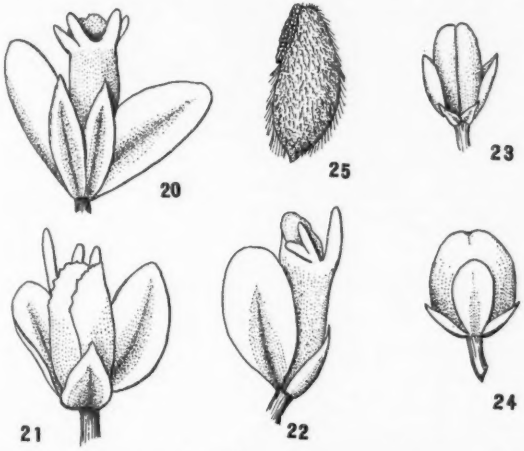
FIGS. 15–19.—Fig. 15, *P. incarnata*, flower, front view; fig. 16, same, flower, back view; fig. 17, same, capsule, back view; fig. 18, same, capsule, side view; fig. 19, same, seed, front view; ×10.

petals have dropped off. The color of the flowers does not turn dark in drying. Characteristic of these species is thus the constant development of a crest upon the keel; the long tubular corolla of *P. incarnata* makes this species very distinct from the others. In the section Chamaebuxus the keel varies from simply rostrate to crested, and in *P. paucifolia* the crest is very conspicuous and plumose; but with regard to the stigmatic structure *P. paucifolia* differs from the species of Orthopolygala as previously described.

VEGETATIVE REPRODUCTION

Of the species examined, *Polygala senega*, *P. polygama*, and *P. lutea* are perennial, the others annuals. There is a very gradual

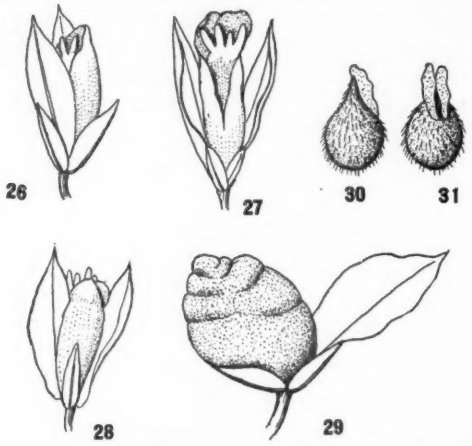
transition from the annual habit to the perennial, and this transition is well exemplified by *P. lutea*. The occurrence of *P. lutea* in the vicinity of Clinton, Maryland, was rather unexpected, because it was known only from a single station near Washington, D.C., a swamp near Suitland, about 10 miles from Clinton; the writer had for several years studied the vegetation in the many swamps near Clinton, but without seeing a single specimen of this characteristic and very conspicuous species. A gravel bank bordering on a sphagnum



FIGS. 20–25.—Figs. 20–22, *P. ambigua*, flowers seen from front, back, and side; ×14; figs. 23, 24, same, fruits, seen from front and side; ×12; fig. 25, same, seed, side view; ×30.

swamp was dug out during the fall some years ago, and the surface, left open, was completely without any vegetation. In the succeeding spring, however, several seedlings appeared, mostly Gramineae, but beside these some small seedlings appeared with epigeic cotyledons, and rosettes of broad, roundish leaves of a light green color. Early in May these seedlings reached the flowering stage, and proved to be *P. lutea*. Among the Gramineae with which it was associated was the very rare *Panicum clutei* Nash, not hitherto observed in this vicinity. How these rare plants had reached this locality seems difficult to explain, unless the seeds had been buried in the ground for several years without losing their vitality. That

grasses may act in this manner is a fact well known, but in regard to *Polygala* it seems very surprising, since the seeds of this genus lose their vitality very soon when collected and kept dry. Nevertheless, I have had the same experience with *P. ambigua*, almost as rare as *P. lutea* in this vicinity, which suddenly appeared in great abundance in a field ploughed over. But returning to *P. lutea*, the seedlings thus reach the flowering stage during the spring, and the leafy rosettes continue to develop leaves and to increase in size. The fruits



FIGS. 26–31.—Figs. 26–28, *P. mariana*, flower, seen from side, front, and back; ×14; fig. 29, same, fruit with one wing; ×14; figs. 30, 31, same, seeds, side and front view; ×20.

matured in July, and from the seeds a second vegetation soon appeared, developing flowers and fruits during the fall, as late as the beginning of November. The rosettes of the plants from May, as well as the later ones, kept fresh during the winter, producing flowers again the succeeding spring. The duration of these plants was observed to extend over three years; thus the species cannot be said to be either annual or biennial, as stated in the manuals. Moreover my herbarium includes some specimens from New Jersey, which show withered stems from the year preceding; thus it seems natural to suppose that the species retains its perennial habit also at stations farther north. The species has no rhizome, but the primary axis per-

sists, increases in thickness, and bears a leafy rosette with axillary buds. The root system consists of several relatively strong, secondary roots, beside the primary, which remains slender, of a light brown color. We have thus in *P. lutea* a plant of exactly the same habit as *Viola tricolor*, described by WITTROCK (10), one of the several analogies existing between these two genera.

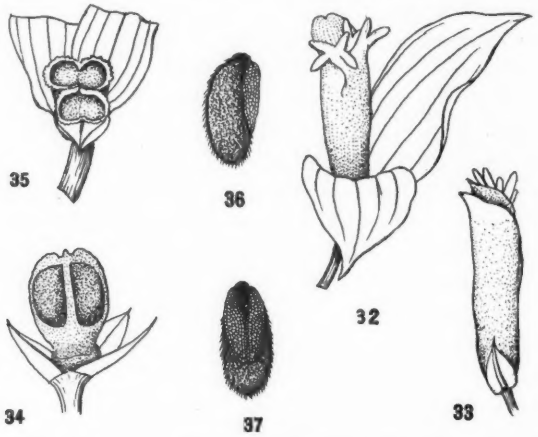
A much stronger perennial habit is represented by *P. polygama* and *P. senega*, in which the primary root persists as a "radix multiceps," increasing in length and thickness, and being crowned by a number of aerial floral stems, which die off at the close of the season, but leave the basal internodes active with their axillary buds. It is a typical pseudo-rhizome: complex of persisting stem bases, but with no rosette of leaves. In *P. polygama* subterranean stems develop from the axils of the lowest leaves, bearing cleistogamic flowers, more fertile than the aerial chasmogamic ones. A similar but less strongly developed pseudo-rhizome recurs in *P. alba*, *P. cymosa*, and the Dahurian *P. tenuifolia*.

Polygala paucifolia is also perennial, but the herbarium material is not sufficient to show the primary development of the deep-seated pseudo-rhizome with its slender roots. The stems do not grow directly upward to the light, but are horizontally creeping beneath the surface for some distance before they develop into erect, flower-bearing stems. These subterranean stems have small scalelike leaves, and they root at the nodes; some of them branch and bear cleistogamic flowers. None of the stems, neither the subterranean nor the aerial, persist for more than one season. This same habit recurs in *P. cornuta* Kell., but the subterranean stems become woody, and the aerial shoots partly persist, thus representing an undershrub. The European *P. chamaebuxus* shows a corresponding habit. In *P. californica*, on the other hand, the root system consists of a large, woody, branched taproot crowned by the persisting stems, thus representing a typical "suffrutex." WARMING (9), who has contributed so much to our knowledge of the biology and structure of Scandinavian plants, described the development of the pseudo-rhizome (radix multiplex) of *P. vulgaris* as follows:

> The thin, decumbent stems proceed from an almost leafless center, and the primary shoot dies off except the basal portion; the cotyledonary buds develop

into decumbent shoots, which ramify, and of which the basal portion persists; the primary root is relatively slender, but persists, and no secondary roots become developed.

Thus in *Polygala* the annual habit passes gradually into the perennial, and from the habit "annual" the transition into "perennial" is well exemplified by *P. lutea*. But none of the North American species develop any further than becoming "undershrubs."



FIGS. 32–37.—Figs. 32, 33, *Polygala lutea*, flower, partly side and partly back view; ×14; fig. 34, same, fruit, side view; ×14; fig. 35, same, base of fruit, carpophore, front view; ×14; figs. 36, 37, same, seeds, side and front view; ×20.

INTERNAL STRUCTURE OF VEGETATIVE ORGANS

ROOT SYSTEM.—The very thin lateral roots of *Polygala lutea*, growing in sandy soil near a creek, have no hairs, but the epidermis is papillose. There is no exodermis, and the cortex consists of three compact layers of large-celled, thin-walled parenchyma. The endodermis is thin-walled, and the pericambium remains intact; the stele is triarch, and the center is occupied by a narrow strand of thick-walled conjunctive tissue. No oil ducts were observed, but many of the cells of the cortex contained a dark granular substance. The primary root shows an early increase in thickness, the pericambium having developed a few strata of secondary cortex but no cork. The stele shows a continuous band of leptome and hadrome in deep rays;

no oil ducts were observed. In the annual species, for instance *P. mariana*, the primary root and its strong lateral ramifications show four to five thin-walled strata of pericambial cork, while all the peripheral tissues from epidermis to endodermis have been thrown off. The stele shows the same structure as the primary root of *P. lutea*, but contains many strata of thick-walled libriform; no oil ducts were observed. A very singular structure is exhibited by *P. senega*, at least in the older roots, as described by the writer (3) in a previous paper. In very young roots the structure is normal, and shows the regular development of all the tissues. In these is a sparingly hairy epidermis, and a cortex of four strata, thin-walled but compact, and with a yellowish substance in some of the cells. The endodermis is thin-walled, and the pericambium surrounds four short rays of hadrome, and four exceedingly small strands of leptome. The vessels and the conjunctive tissue are yellow and thick-walled; no pith is developed. When the roots grow older the structure becomes very much changed, not only on account of the increase in thickness, but also on account of the very irregular increase, so as to make the stele excentric.

By the gradual growth in thickness, the epidermis, cortex, and partly also the endodermis become thrown off, while strata of cork arise from the pericambium. A secondary cortex develops also from the pericambium, and secondary leptome and hadrome begin to form collateral mestome strands, separated from each other by rays of parenchyma. If this increase in thickness due to these secondary tissues were uniform all round the center of the root, we should have a normal and very frequent root structure, but the increase is not regular. The secondary cortex is much thicker on the one side of the root than on the other, and the mestome strands are much broader, that is, they contain a much greater number of vessels in each row than on the other side. Furthermore, the parenchymatic rays are long and narrow on the one side of the root, but very short and enormously broad on the other. It is on that part of the root where the cortical parenchyma is so strongly developed that the characteristic keel becomes formed, when the root is dried. A cross-section of the root thus shows an excentric stele of an elliptic to ovate outline, and a cortex which is thickest outside the broadest mestome

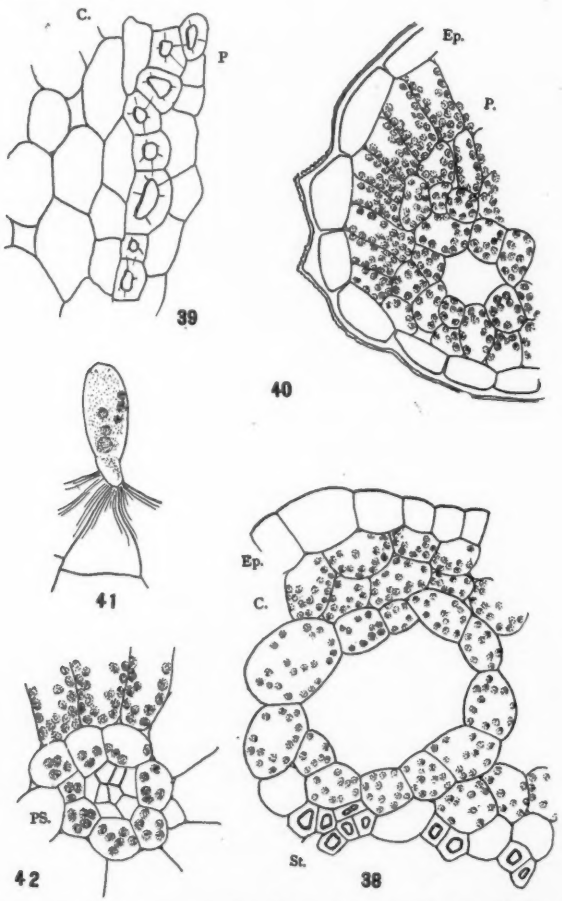
strands. Where the mestome strands are short and the parenchymatic rays very broad, the cortex occupies a very narrow zone of the cross-section. With regard to the minor structure of the various tissues, the cork consists of about four layers of a brown color. The secondary cortex may consist of about twenty layers on the one side of the root, but of only ten or less on the other; the cells are slightly thick-walled, and arranged radially toward the center of the stele. No starch was observed, but a yellow substance occurs frequently in this parenchyma, although not in specialized ducts or reservoirs of any kind. The cell walls of the cortex are also yellow in some places. Small strands of leptome occur in the cortex some distance from the main stele. In the stele is leptome, cambium, and hadrome; the latter containing many wide vessels, and the parenchymatic rays varying in length and width, as just stated; the cells of these rays are often thick-walled. In the vessels and in the adjoining parenchyma is frequently a yellowish, somewhat oily substance, but only in the root, not in the stem and leaves. While thus the most common irregularity in the increase in thickness depends on the one-sided development of the secondary tissues, several other irregularities occur, and have been described by LINDE (4). For instance, the cortex may form two broad wings, one on each side of the root. The stele may be divided into several broad rays of mestome with broad parenchymatic rays; or it may be divided into two separate steles as if two roots had grown together. But whatever irregularities be observed, it seems to be constant that no pith is developed, and that the primordial stele becomes preserved throughout the life of the root. Very characteristic of the root of *P. senega*, however, is the development of a band of leptome strands outside the stele, which is not mentioned by CHODAT. This investigator, on the other hand, observed in *P. obovata* St.-Hil. that the hadrome was divided into a great number of strands of very irregular outline, as in the tuberous roots of *Althaea*, *Scorzonera*, *Daucus*, etc. We have thus in *Polygala* two remarkable cases of root structure, exemplified by *P. senega* and *P. obovata*.

AERIAL STEM.—The stem is cylindric in *P. senega*, obtusely five-winged in *P. incarnata* and *P. lutea*, sharply four-winged in *P. nuttallii*, and sharply five-winged in *P. mariana*, *P. ambigua*, *P.*

curtissii, *P. sanguinea*, and *P. polygama*. The cuticle is relatively thick, but perfectly smooth in *P. lutea*, and also in *P. senega* except where it covers the short curved hairs in the form of pearls; in the other species the cuticle is distinctly wrinkled longitudinally, and especially in the wings. The epidermis is thin-walled in *P. lutea*, and short, unicellular, obtuse hairs are quite frequent; in the other species the outer cell wall of the epidermis is more or less thickened, and especially in the wings, where it is raised so as to form papillae. The lumen of the cells in *P. polygama* is very wide all around the stem. Oil drops were observed in many cells of the epidermis in *P. incarnata*, *P. lutea*, *P. mariana*, and *P. nuttallii*, but not in the four other species, although *P. sanguinea* has oil ducts in the cortex. With regard to the cortex, this tissue is homogeneous in *P. lutea*, *P. polygama*, and *P. senega*, consisting of several, three to six strata of isodiametric cells; while in *P. sanguinea* some palisade cells occur here and there, and a small strand of collenchyma was observed in the sharp wings of *P. polygama*. In the other species examined (Orthopolygala) the peripheral one or two strata of the cortex represent typical palisades, the inner more or less roundish cells. Lysigenous oil ducts were observed in the cortex (fig. 38), and they are surrounded by a ring of parenchyma, the lumen and shape being more or less distinct from that of the surrounding cortex. Their distribution is very regular, corresponding with the outline of the stele, which is pentagonal. In *P. lutea* there are thus ten ducts, five outside the five corners of the stele and five in the spaces between these. In *P. mariana*, *P. nuttallii*, and *P. sanguinea* the five ducts are located outside the five corners of the stele.

An endodermis was observed only in *Polygala senega*, and it was not very distinct; a pericycle, on the other hand, is represented in all the species examined, and it is composed of two or three strata, the peripheral being stereomatic, the inner thin-walled and parenchymatic (fig. 39). The stele itself shows five primary, collateral mestome strands, between which a cambium develops strands of leptome and libriform, but no vessels. This cambium corresponds with the intrafascicular, and the pericycle takes no part in developing the secondary mestome strands. The oil cells, which CHODAT claims to have found in the wood of *P. senega*, were not observed in

this material. The pith is thin-walled and contains neither starch nor crystals of any kind.



FIGS. 38–42.—Fig. 38, cross-section of stem of *P. lutea* showing wide duct in cortex (*c*); *ep*, epidermis; *st*, stereomatic pericycle; ×360; fig. 39, cross-section of stem of same species; *c*, cortex; *p*, pericycle of two strata; stereomatic and parenchymatic; ×360; fig. 40, cross-section of leaf of *P. mariana* showing duct in chlorenchyma, beneath palisade tissue (*p*); *ep*, epidermis; ×360; fig. 41, hair from leaf of *P. lutea;* ×480; fig. 42, cross-section of leaf of *P. incarnata* showing one of the lateral veins with parenchyma sheath (*p s*); ×480.

LEAF.—*Polygala senega* is the only one of the species examined of which the leaf structure is dorsiventral with reference to the stomata, being confined to the dorsal face, and the chlorenchyma being differentiated into a ventral palisade and a dorsal pneumatic tissue. In the other species stomata occur on both faces of the leaf blade, and the chlorenchyma is mostly represented by palisade cells in the ventral as well as in the dorsal part of the leaf.

The cuticle is thin and smooth all over the leaf blade in *P. incarnata*, but wrinkled in the other species, especially where the epidermis is papillose (fig. 40). The epidermis is very thick-walled (the outer wall) in *P. incarnata*, thin-walled in the other species except in the midrib and margins. The stomata are level with the epidermis, and hairs of the type described for the stem are frequent on both faces of the leaf in *P. lutea* and *P. senega*, especially above and beneath the stronger veins. Viewed in superficial sections the lateral walls of the epidermis are nearly straight on both faces of the blade in *P. lutea* and *P. incarnata*, but undulate on the dorsal face in the other species. As already mentioned, the palisade tissue in *P. senega* is confined to the ventral part of the leaf, and the same structure recurs in *P. lutea*, *P. curtissii*, and *P. sanguinea*, but in these the stomata are distributed over both faces of the leaf. In the other species the pneumatic tissue is inclosed by the ventral and dorsal strata of palisade cells. With regard to the occurrence of oil in the leaves, oil drops were found in the epidermis of *P. lutea*, *P. mariana*, *P. nuttallii*, and *P. sanguinea*. Lysigenous oil ducts were observed in the chlorenchyma between the veins, and between each two of these, in *P. lutea*, *P. mariana* (fig. 40), and *P. sanguinea*, while only one single duct was seen in *P. incarnata*. No oil drops nor ducts were found in *P. curtissii*, *P. ambigua*, *P. senega*, nor *P. polygama*, neither in the stems nor in the leaves.

The mechanical tissues, stereome and collenchyma, are almost absent; in *P. senega* there are a few collenchymatic cells beneath the dorsal epidermis, and in *P. ambigua* the midrib has a small support of rudimentary stereome on the leptome face. Water storage tissue is also poorly represented in the dorsal keel of *P. senega*, *P. polygama*, and *P. lutea* (stem leaves). The median mestome strand is crescent-shaped in cross-section, and more or less imbedded in the

chlorenchyma. The lateral veins (fig. 42) are generally very thin, and surrounded by thin-walled, green parenchyma sheaths.

PETIOLE.—There is a distinct but relatively short petiole in *P. senega;* it is crescent-shaped in transverse sections, and hairy like the stem and leaf blades. Many of these hairs are borne on small cushions of epidermal cells, however, and are frequently curved. The cuticle is prominently wrinkled, and the epidermis is thick-walled. A collenchymatic tissue occurs on the ventral face beneath the epidermis. The single mestome strand is surrounded by a large colorless parenchyma, but no stereome is represented.

These species of *Polygala* thus exhibit several peculiar structures in the roots, as well as in the stems and leaves. In the root of *P. senega* the presence of a band of leptome strands outside the stele may be seen. A yellowish, oily substance was observed in the root of this species, in the secondary cortex, also in some of the vessels and the adjoining parenchyma, while no oil was found in the leaves or stems. In several of the other species oil drops were found in the epidermis of stem and leaves, and lysigenous oil ducts were also observed in several species, in the leaves between the veins, in the stem outside the corners of the pentagonal stele (in cross-sections), and sometimes also in the spaces between. The structure of the cortical parenchyma varies from homogeneous, with all the cells isodiametric, as in *P. lutea*, *P. senega*, and *P. polygama*, to heterogeneous, where the peripheral strata represent palisade, the inner ordinary parenchyma cells, and this structure occurs in the other species. A similar deviation was noticed in the leaves, where a ventral palisade tissue and a dorsal pneumatic occurs in *P. senega*, *P. lutea*, *P. sanguinea*, and *P. curtissii;* while in the remaining species the pneumatic tissue is inclosed by ventral and dorsal strata of palisade cells. The distribution of the stomata does not correspond altogether with these leaf structures, for *P. senega* is the only species showing a truly dorsiventral structure as to chlorenchyma and stomata; while in all the other species the stomata occur on both faces, whether the chlorenchyma shows a bifacial or centric structure, as just described. The mechanical tissue, stereome, is almost entirely absent from the leaves, but in the stem it forms a more or less closed sheath (pericycle) interspersed with thin-walled parenchyma surrounding the

stele. In several species the pericycle is composed of two or three strata: the peripheral stereomatic, the inner parenchymatic. The increase in thickness of the stele depends on an interfascicular cambium, and not upon any activity on the part of the parenchymatic portion of the pericycle.

Summary

According to the material of North American species of *Polygala* here examined, it would seem that some of the subsections of Orthopolygala proposed by CHODAT are too heterogeneous to be considered really natural. For instance Incarnatae, comprising types so distinct as *P. incarnata*, *P. cruciata*, *P. mariana*, *P. sanguinea*, etc., are characterized by "stylus ovario longior, filiformis," which certainly applies only to *P. incarnata*. Moreover, the floral structure of this species (figs. 15–18) is very distinct from that of the others. The structure of Decurrentes *P. lutea:* "sepala cum pedicellis concrescentia i.e. pedicelli alati" was not to be found in the material here examined. The combination of *P. senega* and *P. polygama* into one subsection seems strange, when we compare the style structure and the different habit of these species, notably the cleistogamic flowers of the latter.

These four species, *P. incarnata*, *P. lutea*, *P. senega*, and *P. polygama*, are so distinct from all the others that they may be classified as monotypic subsections. With reference to *P. lutea*, the perennial habit by means of a persisting rosette of leaves would represent one good subsectional character; the floral structure a second, because of the development of a carpophore; and the wings, persisting for some time after the fruit has dropped, represent characters of greater importance than simply specific. The characters exhibited by the internal structure are merely specific, for instance, the centric leaf structure and the cortical parenchyma being homogeneous in some species and heterogeneous in others; as well as the occurrence of oil ducts in some species and its total absence from others. The peculiar secretions which VESQUE found in the chlorenchyma of several Brazilian species may be referable to oil, which CHODAT observed in the leaves of many South American species, but contained in lysigenous ducts. The crystalline appearance of the secretion found by

VESQUE was evidently due to the preparation of dried material. In the living plants examined the oil was perfectly normal, and sometimes quite abundant. But while CHODAT never observed these oil ducts in the stem of any of the South American species, they are nevertheless well represented, and very regularly indeed by some of the North American, as here described. The occurrence of secretions in *Polygala*, in species of limited geographic distribution in North and South America, thus represents an analogy to *Viola*, of which several species peculiar to Chile and eastern North America contain secretions in the roots, stems, and leaves.

Finally, with reference to epharmonic characters, according to my observations *Polygala* shows only a very few that may be identified as such. This is especially true of *P. senega* from woods and dense thickets, where the leaves show a strictly dorsiventral structure, while in the other species the stomata are distributed over both faces of the blade, whether the structure of the chlorenchyma is centric or dorsiventral. And this varied structure of the chlorenchyma accompanied by the constant distribution of the stomata is exemplified by species inhabiting more or less dry or moist sandy soil in the open, and very frequently associated with each other. While the leaves are destitute of mechanical tissue, such is present in the stems of all the species examined, but only in the form of a pericycle, and of a single stratum. The presence of oil ducts may hardly represent an epharmonic character, since it is not connected with any particular environment.

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DEVELOPMENT OF NORMAL AND DIVERGENT PLASTID TYPES IN ZEA MAYS

CONWAY ZIRKLE[1]

(WITH PLATES X–XII)

Introduction

The transmission of plastid abnormalities has been shown to be conditioned, both by chromosome-borne genes, and by certain elements in the maternal gamete as yet unidentified. This second type of inheritance is thus far unique in that it alone appears to be non-Mendelian. The fact that, with the exception of *Pelargonium zonale*, the male gametes are unable to influence these maternally inherited deficiencies has been accepted by many workers as presumptive evidence that the material basis of this inheritance is located in the cytoplasm. While the exisiting genetic data permit an interpretation which would assign a nuclear basis for this inheritance, as DEMEREC (6) has pointed out, the fact that all known cases of maternal inheritance are concerned with plastid abnormalities strengthens the hypothesis that there is a cytoplasmic basis for their transmission.

The chloroplasts themselves would furnish a ready-made vehicle for this transmission, if it could be shown that they originated only through the division of pre-existing plastids which persist through all stages of the sporophyte and of the female gametophyte. The existence of such self-perpetuating chloroplasts can easily be demonstrated in certain algae and archegoniatae. In the higher plants, however, it can be shown that no such conspicuous cell organs as chloroplasts exist in certain stages in the life cycle. They must either originate *de novo* or develop from some self-perpetuating primordia. Such development from the primary meristem to fully differentiated tissue has frequently been traced in a number of species in both living and fixed material. No general discussion or review of the literature will be essayed here, as this has been done

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in some of the more recent papers, as those of EMBERGER (7), RANDOLPH (14), KASSMANN (8), KIRBY (9), etc.

In any study of abnormal plastid types certain questions naturally arise. In what stage of the development can the abnormality first be recognized? Is the deficiency due to arrested development or to the degeneration of either partially or fully developed plastids? Is it possible to distinguish the primordia of abnormal types from those of the normal, and thus to trace directly the evidence of abnormality through the life cycle?

Zea mays has many advantages for an investigation of this kind. Numerous types of chlorophyll deficiency are known, whose specific conditioning genes have been located. These range from complete albinism to types which appear quite normal in the mature plant. Many different kinds of variegations are included, in some of which both normal and abnormal chloroplasts exist in the same plant. There are in addition three known cases of maternally inherited deficiencies.

The present paper contains a description of the normal plastid development, supplementary to a previously published account (16), and of the development of two albino stocks, a maternally inherited deficiency, a yellow-green lethal, an aurea, an argentea, and an expallescent-virescent stock.

This investigation of plastid primordia is limited to fixed material, although mature and developing plastids were examined in living cells. While none of the fixatives used preserved the exact form of these primordia, their fixation images had been previously checked with living tissue (ZIRKLE 16), and thus in general it was possible to allow for certain known artifacts. There were, however, certain cases of unexplained variations in the fixation images. While it is possible that these may be due to an erratic behavior of an otherwise dependable fixative, the evidence indicates, as will be pointed out later, that they are caused by variations in the tissue fixed. The study of fixed primordia is, in a way, a necessary evil. Their small size and their index of refraction make it extremely difficult to differentiate them from ergastic material in uncleared cells. Their reaction to vital stains is highly non-specific, and, as RANDOLPH (14) reported, of little value in their study. At present their identifica-

tion by their highly specific reactions to fixation is more certain (KIRBY 9, ZIRKLE 17, 18), as well as by their clear-cut staining properties after being properly fixed and mordanted. Of course, under these conditions failure to find differences between those primordia which develop into normal plastids and those which develop into abnormal ones is in no way conclusive.

As the investigation was limited to plastids and their primordia, no attempts were made to preserve the various mitotic figures. The most satisfactory fixing fluid for this purpose was a modification of Erliki's potassium bichromate copper sulphate mixture. Erliki's fluid unmodified did not penetrate well. The leaves have such an impervious waxy cuticle that they could be fixed only by drawing the fluid up through the vascular bundles. The substitution of ammonium bichromate for the potassium bichromate overcame the difficulties of penetration but caused the outer layers of cells to be badly over-fixed. The most successful proportions for the fixative were:

$K_2Cr_2O_7$	1.25 gm.
$(NH_4)_2Cr_2O_7$	1.25 gm.
$CuSO_4$	1.00 gm.
H_2O	100.00 gm.

This mixture gave essentially the same image to both outer and inner layers. The fixation images of various other fluids will be described later. The only stain employed was Haidenhain's haematoxylin.

For the sake of definiteness, certain terms will be used in a strictly limited sense. The term mitochondria will include all rod-shaped, thread-shaped, or granular cytoplasmic inclusions preserved by bichromates on the basic side of the range pH 4.2–5.2 (the exact point depending upon the specific cation), and destroyed by those on the acid side of this range and by mixtures of bichromates and acetates. The term plastid will include only those cell organs which contain starch or chlorophyll.

NORMAL PLASTID DEVELOPMENT

In the growing point of the root the plastid primordia are mitochondria. Their shape and size are constant with a given fixative, but they vary greatly when fixed with different fluids. When

the active fixing agent is formaldehyde they are thread-shaped; the same is true when they are fixed with ammonium bichromate. When fixed with potassium bichromate, copper bichromate, etc., they are rod-shaped. When the alkaline earths furnish the cation of the bichromate fixative, they tend to be granular or to form chains of granules. Lithium bichromate fixes them as distinct spheroids. While there is a slight variation of the size and shape of the mitochondria within a single cell, it is nowhere great and there is nothing to indicate two distinct types. There is no grading off in size to the border-line of visibility. The mitochondria elongate and divide transversely; and if any originate *de novo*, they must appear suddenly fully formed. There is a constant difference in the mitochondria in the different cells of this region regardless of the fixative used. They are larger and heavier in the epidermis and cortex and slenderer in the procambial cells and in the central cylinder. Constant as this difference is, however, it may be due entirely to an uncontrolled factor in the fixation. The fluid which first reaches and fixes the inner cells is more dilute than that which fixes those on the periphery, and considering how markedly the mitochondria react to slight differences in the fixation, this variation in size may be more apparent than real.

Slightly farther from the tip in the region of elongation the mitochondria are much more stable, fixing generally as rods; although with the bichromates of the alkaline earths they still tend to fix as chains of granules. With lithium bichromate, however, they are rods; and therefore by tracing a single line of cells back from the tip it is possible to trace the development of plastid-like bodies into distinct mitochondria, a reversal of the usual course of development.

In the region of differentiation two distinct sizes of mitochondria are found in each cell (fig. 5). These two sizes soon become quite distinct with no transitional stages between them. They are clearly fixed with the ammonium potassium bichromate mixture. The development of the larger ones can easily be traced until they are distinct plastids. The smaller apparently undergo no further change, and persist in the same form in cells which contain mature chloroplasts.

The presence of large starch grains has been reported frequently

in the cells of the root cap. These grains are contained in mature plastids and occur in fully one-half of the cells of the cap. In the younger portion of the cap, the meristematic cells are in intimate contact with the apex of the root proper, and contain mitochondria of the same size and shape as those in the dermatogen, but no plastids. Between these two regions are cells which contain transitional stages. The whole development from primordium to mature plastid thus takes place within the range of a few contiguous cells, and all of the stages can be shown within a single microscopic field. The transitions from mitochondria to small plastids are shown in fig. 2.

The plastid primordia in the growing point of the stem are either mitochondria or minute plastids. As these two categories are but developmental stages of a single cell organ, the fact that either may occur in a given tissue indicates that plastid development is to a certain extent independent of the development of the plant as a whole. Just what growing conditions in the stem tip are prerequisite for the presence of either form is uncertain. There is no uncertainty, however, about the existence of either of these two stages. Fixed with ammonium potassium bichromate, the mitochondria are minute rods measuring 0.25 by 0.75 μ. The same fixative sometimes preserves the primordia as small spheres both in the stem tip and in the root tip (fig. 7). The plastids, small hollow spheres, can be identified by the occurrence of a minute starch grain 0.5 μ in diameter in their central vacuoles. These grains become very distinct when the balsam mounts are examined in polarized light.

The cells of the epicotyl immediately posterior to the convex growing point of the stem contain numerous small plastids. In the cortex four or five layers farther back the plastids are mature. Here therefore the development from primordia to plastids is completed within the range of as few cells as it is in the root cap. The epicotyl is apparently an important storage organ, as it contains numerous plastids all of which are full of starch. The one exception is the long procambial cell where the plastids do not develop as in the cortex but rather seem to degenerate. They become vacuolate, and their stainable substance collects in groups of two, three, or four peripheral granules (fig. 3). Later the plastid breaks down but the

granules still tend to remain in groups of two, three, four, or five, four being the usual number.

If these granules can be classified as mitochondria, then they are the only mitochondria in these cells, since there are no other cytoplasmic inclusions with the fixing properties of such bodies. The ultimate fate of the granules is uncertain. It seems probable that they elongate into bodies which can be classified definitely as mitochondria; for the cells of young vascular bundles developing from these procambial cells are filled with mitochondria (fig. 4) which, farther back in older bundles (fig. 6), become transitional forms between mitochondria and plastids. Here the rods increase greatly in size and become vacuolate, each vacuole containing a single starch grain. The branch roots, which emerge from the lower nodes of the stem, and the prop roots, which grow down from the next higher nodes, contain mitochondria in their apical cells. The lineage of these cells can be traced back to cells near the growing point of the stem which contain numerous small plastids but no mitochondria. Thus it seems that the usual course of development from mitochondria to plastids is often reversed.

The primordia of chloroplasts in the chlorenchymous tissue of the embryonic leaves in young seedlings are minute plastids. They fix as small hollow spheres; and in normally growing green plants each contains a single starch grain in its central vacuole. There is little variation in size in these plastids within a single embryonic leaf until after the tip of the leaf has emerged, although there is a distinct increase in their size in the successive leaves from the stem growing point outward, until in the tip of the coleoptile they are almost mature. The coleoptile differs in some respects from the other leaves. While the cells in its tip contain plastids, those in its base contain mitochondria, much larger than the plastids in the inner leaves. In every leaf tip, as RANDOLPH reported (14), there are plastids, smaller in the younger leaves and larger in the older. The epidermal cells at the base of the embryonic leaves contain mitochondria.

Different cells in the mature leaf have very different kinds of plastids, as is shown in fig. 1. In the phloem the plastid primordia remain very small and fix as mitochondria. In the chlorenchyma

between the bundles they remain relatively small, and are typical chloroplasts, seemingly solid bodies containing starch in their central vacuoles only when conditions have been favorable for photosynthesis. In the cells immediately surrounding the bundles the chloroplasts are much larger and are especially modified for the storage of starch. Each plastid in these cells contains a centrally located starch grain (fig. 1). When fixed with ammonium potassium bichromate, the pores leading into their central vacuoles (ZIRKLE **15**) are enlarged and may easily be seen. When the plastid is so oriented that the flat surface is uppermost, the pores appear to be round, but when it is seen from the side they appear to be slits (fig. 1). This would indicate that the pores, somewhat concentrated on the flatter surfaces of the plastid, lead directly into the vacuole, yet do not point to the plastid's center.

MENDELIAN CHLOROPHYLL DEFICIENCIES

ALBINOS

DEMEREC (**3**) showed in his study of white seedlings that albinism could be caused by many different genes. There is nothing to indicate that the development of plastid primordia is alike in all these stocks. RANDOLPH (**14**) found plastid primordia in all of those cells in the albino which in normal seedlings contain green plastids, and even found green chloroplasts in the extreme tips of certain albino leaves. MILES (**13**), on the contrary, reported a complete absence of plastids in an albino stock, and figures no primordia. MILES' results are of little value, however, as he fixed the leaves he examined in a modification of Carnoy's fluid which destroys all plastid primordia. The investigations of two different albino stocks are recorded here.

In stock I, supplied by Dr. A. J. MANGELSDORF, the albinism is due to a single recessive gene which has not been located. It is a very vigorous, fast-growing stock. The albino seedlings normally reach a height of about 8 inches before they stop growing and die. When not exposed to direct sunlight they even reach a height of from 12 to 15 inches. No trace of green was ever observed in any of the albino seedlings.

Plastid development in the roots of these albinos is normal in

every way, and indistinguishable from that in the green seedlings. The same is true for the plastid primordia in the growing point of the stem, and for the plastid development in the cortical cells of the epicotyl in young seedlings 3 or 4 inches high. It should be emphasized that the cells in the cortex are crowded with mature plastids, each of which is full of starch. The procambial cells are in every way normal, and the vascular bundles contain numerous transitional stages between mitochondria and plastids (fig. 6). In older seedlings which have reached the maximum height, however, the condition of the plastids in the now somewhat elongated epicotyl is quite different. Large starch-filled plastids still exist, but their numbers are so diminished that it appears as if their multiplication had not kept pace with the tissue's growth. In many of the cortical cells in the upper part of the epicotyl there are no plastids; instead one finds gigantic mitochondria 1 μ in diameter and from 20 to 50 μ in length. It would seem that the seedling has lost the power of maturing plastids.

Plastid development in the leaves is very abnormal. The primordia in the leaves of the young seedling appear normal except for their lack of pigment. As growth progresses, however, they diverge more and more from the type. Distinct immature plastids 2 μ in diameter occur only along the edges of the leaf tips and in the terminal cells of the midrib. Some few cells in the bases of the leaves contain mitochondria. The greater part of the leaf, however, contains no recognizable cytoplasmic inclusions, although the cytoplasm of some of the cells is slightly granular. If any primordia are present they are very degenerate, and are not fixed by the fluids which ordinarily preserve mitochondria.

It is important to note that the state of the plastids in the albino seedlings is not due merely to their failure to complete development, but has an additional cause in a degeneration which ensues after they reach certain developmental stages. Except for the absence of pigments, the plastids appear entirely normal in the very young seedlings, and some leucoplasts actually mature in the epicotyl. In the older seedlings the only cells in the leaves which contain recognizable plastids are those in the tips which were formed either in the embryo or when the seedling was very young. This deficiency

is due to the presence of a single homozygous recessive gene. The mother plant was of course heterozygous for this gene and had normal appearing green plastids. If the genes of the mother plant influence the plastid development in the embryo, or help to catalyze some substance necessary for complete plastid development which is stored in the seed, there is a ready explanation for the early development and later degeneration of plastids in the homozygous recessive albino seedlings. The stoppage of development and degeneration would then be coincident with the exhaustion of this substance.

It has generally been assumed that the death of albino seedlings is due to starvation; unable to synthesize carbohydrates they can live only until the food stored in the seed is exhausted. It is interesting to note that death first occurs in the leaves where there is no stored food, yet the whole seedling dies while the epicotyl still contains some stored starch. Obviously death is not due entirely to a lack of carbohydrates.

The albino seedlings are just as phototropic as the normal green type. A tray containing both green and albino plants was illuminated from one side. Both types of seedlings grew toward the light, and no difference in kind or amount could be noted in the reactions of the two groups.

Stock II was supplied by Dr. W. H. EYSTER and described by him as often producing green stripes and being sometimes viable. Under growing conditions in the greenhouse of the Bussey Institution this was a very feeble stock, the seedlings rarely reaching a height of 3 inches. They showed no trace of any green tissue and were apparently pure albinos, although it would perhaps be more accurate to describe them as extreme cases of virescence. Their plastid development was normal except for a complete lack of color. Even just before death there was apparently an adequate supply of minute plastids in all of the cells in the mesophyll (fig. 9).

GREEN-YELLOW LETHAL

This stock was supplied by Dr. W. H. EYSTER. The population obtained consisted of approximately three parts normal plants and one part of greenish yellow seedlings, which latter seedlings died

after reaching the height of about 6 inches. As has been found in every case thus far, no differences could be observed in the plastid primordia between the deficient and the green seedlings. The small plastids in the young leaves seemed normal. When they reached a fourth of the mature size, however, further development ceased and they began to degenerate (fig. 8). This degeneration sometimes precedes the death of the plant by several days. The plastids become ragged, tend to fragment, and very darkly staining granules appear about them in the cytoplasm. The cells are full of débris when death occurs.

AUREA

This stock was also furnished by Dr. W. H. EYSTER, who has not as yet published the genetic data.[2] The deficiency is due to a gene in chromosome I. Seedlings showing the character are a golden yellow, and are much lighter in color than the greenish yellow lethal just described. EYSTER reports that the aurea seedlings have a full complement of xanthophyll, carotin, and chlorophyll b, but little or no chlorophyll a. The seedlings are very vigorous and grow rapidly, and, when they reach the height of 18 inches the tips of their leaves begin to turn green. The mature plants are green and are but little lighter than those which are normal.

The plastids, except for pigment content, seem completely normal throughout the entire plant. They are especially large, well developed, and numerous in the yellow leaves. When the seedlings are 6 inches high, the stage where the green-yellow lethals die, the diameter of their plastids is three times that of the latter. It should be emphasized here that the death of certain lethal types, which are identified by their chlorophyll deficiency, may be caused by factors much less conspicuous than the lack of pigment. The two types just described are cases in point. The stock which had the greater amount of pigment but defective plastid development was lethal; the stock which had less pigment but well developed plastids was viable.

[2] At the time of reading proof these data have been published: EYSTER, W. H., Five new genes in chromosome I in maize. Zeitschr. Indukt. Abstam. Vererb. **49**: 105–130. 1929.

ARGENTEA

This chlorophyll pattern, due to a gene in chromosome I, was also supplied by Dr. W. H. EYSTER (genetic data not published). The seedlings, which at first appear to be a type of virescent, vary greatly in their amount of pigment, and likewise show great variation in rate of growth, the darker seedlings growing more rapidly. The first two leaves of the average 6-inch seedling are white except for a green midrib and border. The third and fourth leaves show more chlorophyll. In the half-grown plant the first four leaves and the tip of the fifth show many white or light green stripes, which, alternating with darker tissue, give the whole a silvery sheen. The other leaves are usually normal, although in extreme cases the whole plant will have very minute light green stripes.

The influence of this gene makes itself felt during a single limited period in the growth of the seedling. It does not influence the first foliage tissue, as is shown by the midrib and the tips of the first leaves being normal green. It delays or inhibits plastid development in the first four leaves and the tip of the fifth. When the bases of the fifth and later leaves develop, the gene usually ceases to influence plastid development, as this tissue appears normal.

Plastid primordia are entirely normal in this type. Their development in certain leaf tissue is inhibited or prevented from going beyond a certain point. In the fine light green or white stripes the plastids rarely reach over one-third of their diameter in the darker stripes. This is especially evident in the cells which surround the vascular bundles (fig. 13).

EXPALLESCENT-VIRESCENT

This aberrant type is segregated from a stock supplied by Dr. A. J. MANGELSDORF. It is due to a single recessive gene which has not as yet been located. When these seedlings first emerge from the ground they cannot be distinguished from their normal sibs. After 3 or 4 days, however, they lose most of their color. The first leaf becomes pale green while the second, third, and fourth leaves become white except for green midribs and borders. There is a great variation in the amount of pigment in the various plants at this stage. Some of the lighter plants die, while the darker ones

grow only about half as fast as a normal seedling from the same ear. Gradually these plants acquire chlorophyll. The first leaf becomes a bright green while the next few leaves acquire green stripes. Leaves subsequent to the fifth are essentially green, although they contain several white stripes averaging 0.25 inch in width. The mature plants contain these stripes, although there is great variation as to their number and distribution.

The primordia here, as in all other types, are normal, as are also the earlier stages of development. There succeeds a period in which development is checked and apparently reversed for a time. Normal development then proceeds to maturity. Many plastid types can be obtained from the different leaves of the same plant and from the same leaf at different times. Fig. 14 shows half-grown plastids in the third leaf when it is developing green stripes. The granular cytoplasm shown is often found at this stage. It is worthy of record that in every abnormal type here investigated the deficiencies do not appear until after the seedling has been growing for a time independently.

Maternal inheritance chlorophyll deficiency

This stock was sent in the spring of 1926 by Dr. W. H. Eyster, who has determined but has not yet published on the genetic behavior. It is apparently a third instance of maternal inheritance in *Zea*. Three types of seedlings were produced by the segregating seeds: light green seedlings, which die when they reach the height of about 6 inches; seedlings with longitudinal light and dark green stripes, most of which mature and produce all three types of offspring; and normally green seedlings, which produce only normal offspring. This maternally inherited abnormality is quite different from the one described by Randolph (14) and Anderson (1), and corresponds in all essential details with that described by Demerec (6).

The striped seedlings show a great variation in the amount of light and dark tissue which they possess, ranging from the entirely light seedlings to those entirely normal. The lighter variegated ones live but a little while after their completely light green sibs die. In the later stages of development their growth is very abnormal. Stem growth stops and the leaves, without increasing in width,

elongate greatly and become pendent. It is no unusual sight to see one of these seedlings 6 inches in height with leaves over a foot long. Before death occurs, the lighter portions of these leaves become much paler than in the seedlings, which possess no dark tissue. When the light and dark areas are about equal the seedling is practically as vigorous as those normally green.

In the very young plant, when the light and dark stripes can first be recognized, the light area is the color of the pale green seedlings, and the color of the dark area is normal. As growth progresses the difference in color of the two regions becomes more pronounced. The darker regions become more intense while the light regions fade. All stages in stripe formation can be observed in plants as they reach the tasseling stage. The stripes on the older leaves at the bottom of the plant may be dark green and cream colored. The stripes in the tips of the upper leaves may be light and dark green. If these stripes be traced toward the leaf bases, it can be seen that their shades approach each other until on reaching the bases their colors become identical, and no stripes whatever are visible. The shade of the leaf base is that of normal young chlorenchymous tissue.

No cytological difference can be observed in the plastid primordia which develop into the normal and divergent plastid types. The mitochondria in the root tips and the plastids and mitochondria in the stem growing point and epicotyl are the same in all three types of seedlings.

The differences in plastid development must be traced in the leaves. It is obvious that the difference between the plastids in the light and dark areas depends upon the age of the tissue. In that region of very young leaves where the stripes can just be recognized, no difference whatever can be observed in the individual plastids of the two regions. In both living and frozen sections the plastids are the same size and color. In fact the two regions are much more distinct macroscopically than in microscopic sections. The earliest distinguishable differences in shade are due to the relative number of plastids and their arrangement in the cells (fig. 10). This difference is particularly clear in the chlorenchymous cells contiguous to the vascular bundles. In the darker stripes these cells contain numerous plastids arranged around their peripheries; in the lighter stripes they

contain few plastids. In tissue a little older, differences can be observed in the plastids themselves. Their development becomes retarded in the lighter stripes, so that these are somewhat smaller and lighter than in the darker portions of the leaf. A cytological difference in the types can here be demonstrated. When the tissue is fixed with ammonium potassium bichromate, stained in haematoxylin and destained, the aberrant plastids lose their color before the normal ones. This is shown in fig. 11. In still older tissue the plastid differences have increased. In the lighter areas the plastids have degenerated, while in the darker they reach mature size. The degenerating plastids fragment and the fragments disintegrate. When the light stripe is cream colored its cells contain nothing recognizable as plastids (fig. 12).

It is of distinct theoretical importance to determine whether there are but two kinds of chloroplasts in the striped seedlings or whether transitional stages exist on the border-line between the stripes. It is also of importance to know whether more than one kind of plastid can be recognized within a single cell. This problem is complicated by the fact that different types of plastids exist in normal leaves. An investigation of very young tissue does not determine the point, for the variation in plastid size from cell to cell exists in unvariegated leaves. In older tissue the answer is plain. There are two separate and distinct types of plastids and no transitional stages. The borders of the stripes, however, are seldom clear-cut and precise. The plastids in the cells surrounding the vascular bundles are apparently quite independent of those in the mesophyll between the bundles. Frequently the cells contiguous to the bundles have normal plastids, while the mesophyll cells which surround them contain no recognizable plastids (fig. 12); on the other hand, the latter cells may contain normal plastids in regions of the leaf while those which surround the vascular bundles contain the aberrant type (fig. 11). At no time were both kinds of plastids observed within a single cell, and frequently cells containing normal plastids occurred well into the lighter regions of the leaf (figs. 11, 12). This localization of a single plastid type to each cell is in keeping with what ANDERSSON (2) found in *Adiantum cuneatum*. The questions as to whether or not these two kinds of plastids are conditioned by

two distinct categories of primordia, which are, however, morphologically alike, and whether two types of primordia exist in a single egg, cannot at present be answered.

Summary

Plastid primordia in the root tips of *Zea mays* are mitochondria. In the root cap their development into mature plastids can be traced within a single microscopic field. In the region of differentiation of the root proper two distinct types of mitochondria occur, the larger of which develops into plastids while the smaller apparently undergoes no further change. In the stem growing point the primordia are generally minute plastids, that is, hollow spheroids containing starch grains 0.5 μ in diameter in the central vacuoles, although occasionally they are mitochondria. Back from the growing point of the stem in the epicotyl, the primordia quickly develop into plastids. In the procambial cells plastid-like bodies can be observed to fragment, and there is some evidence that these fragments develop into mitochondria. In the vascular bundles many transitional stages can be observed in the course of development of mitochondria into plastids. In the leaves the primordia develop into mitochondria in the phloem, into large starch-containing plastids with prominent pores in the cells immediately surrounding the vascular bundles, and into smaller, apparently denser plastids in the chlorenchymous cells between the bundles. In all aberrant types investigated, the plastid primordia were normal so far as could be observed, the divergent types being due to (1) a delayed development, (2) a stoppage of the development, and (3) a stoppage of development followed by degeneration. In the first albino stock investigated the mode of development in the young plastids suggested an influence exerted by the genetic constitution of the mother plant. A disintegration of the partly developed plastids in the leaves of this stock occurred in all except the earliest-formed cells of the foliage tissue, that is, the tips of the first, second, and third leaves. The second albino stock was evidently an extreme case of virescence. All of the mesophyll tissue of the leaves contained minute plastids. Both albino stocks died while starch was still stored in the epicotyl, indicating that starvation was not the primary cause of death.

The divergent plastids in the maternal inheritance stock developed as in normal tissue until they were about half-grown. The first signs of abnormality were not in the plastids themselves but in their number and arrangement within the cells. In tissue of half-grown leaves, a distinct size difference could be observed between the plastids in the light and the dark stripes. In old tissue the plastids in the lighter areas degenerated and disappeared. Mature leaves showed but two kinds of plastids, and contained no intermediate forms on the border-line of the two regions. In the green-yellow lethal stock the plastids degenerated after reaching one-fourth the normal diameter. In "aurea" plastid development was quite normal except for the pigment content. In both the "argentea" and the expallescent-virescent stock the abnormality was due to a checking of plastid development early in the life of the seedling, and to the later normal plastid growth. In general, the abnormal period was between the formation of the tip of the first leaf and the formation of the base of the fifth leaf. Both stocks were very variable.

The writer wishes to express his thanks to Professor W. H. EYSTER and Dr. A. J. MANGELSDORF for their kindness in giving him the aberrant plastid types, and he is especially grateful to Professor E. M. EAST for his help and criticism during the course of the investigation.

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EXPLANATION OF PLATES X–XII

The photographs were made from slides stained with Haidenhain's haematoxylin. No counter stain was used. All were fixed with the ammonium potassium bichromate modification of Erliki's fixative.

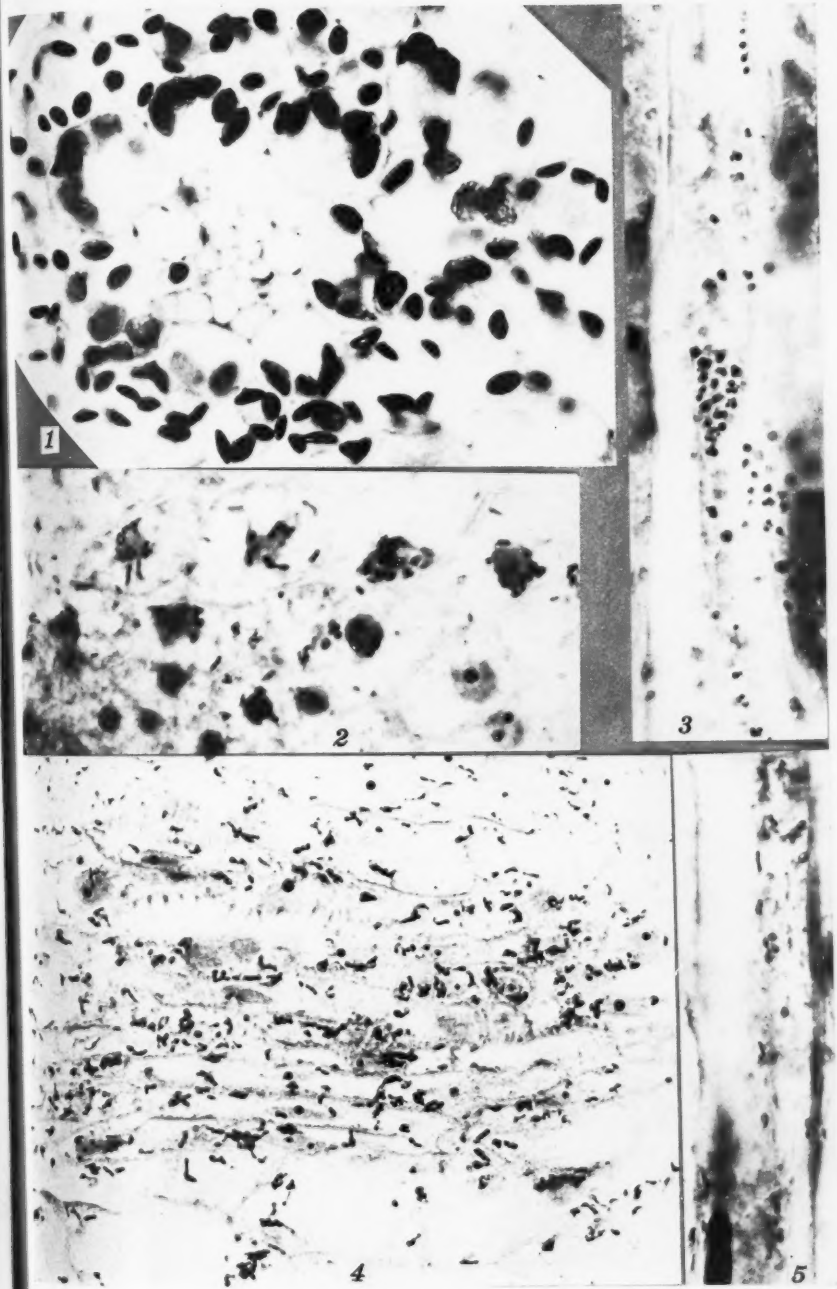
PLATE X

FIG. 1.—Cross-section of leaf of normal green plant; three end products of development of plastid primordia are shown: (1) mitochondria in vascular bundle, (2) large starch-containing plastids with prominent pores in cells surrounding bundle, and (3) smaller typical plastids in mesophyll; ×1000.

FIG. 2.—Longitudinal section of root cap showing mitochondria (on left) developing into plastids (on right); ×750.

FIG. 3.—Longitudinal section of procambial cell in epicotyl showing plastids breaking up into granules; ×1500.

FIG. 4.—Longitudinal section of vascular bundle in epicotyl showing mitochondria and transitional stages; ×400.



ZIRKLE on ZEA MAYS

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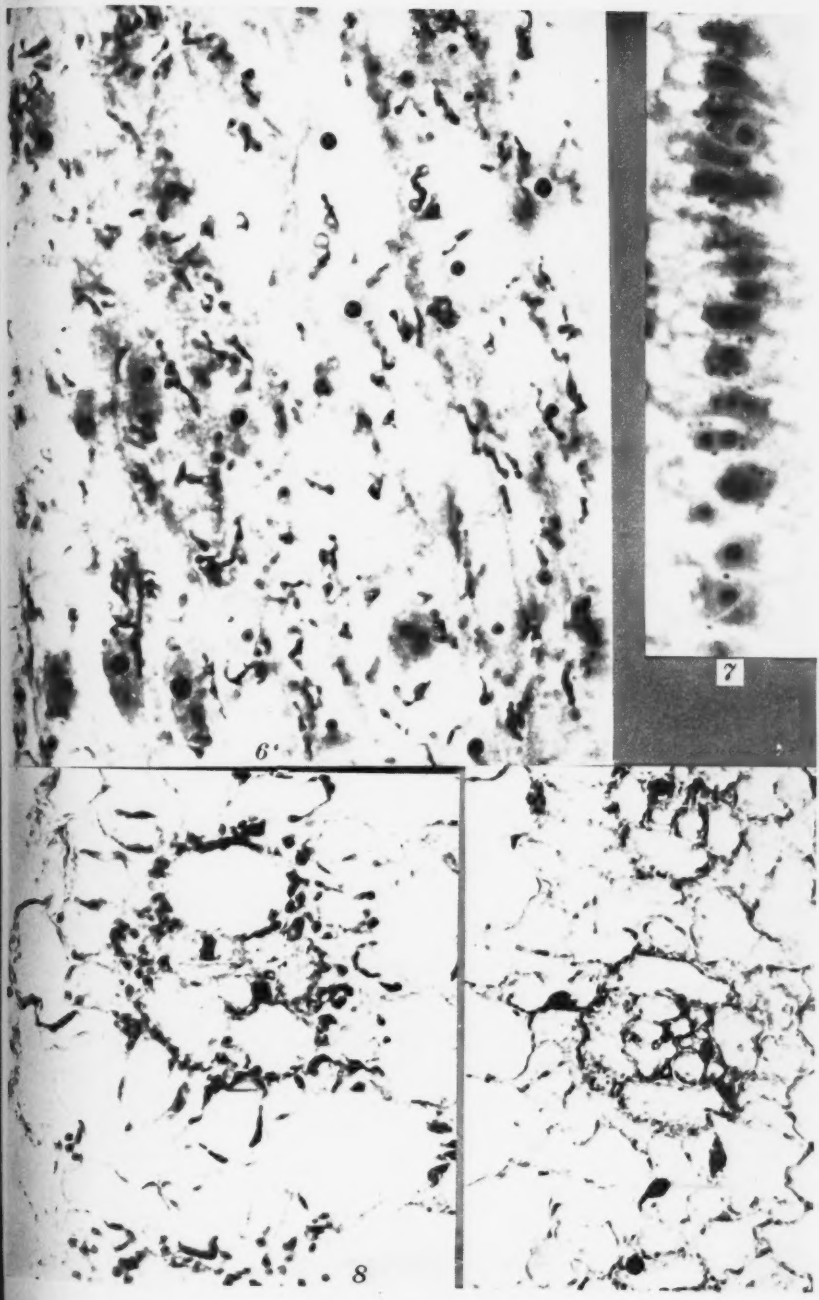
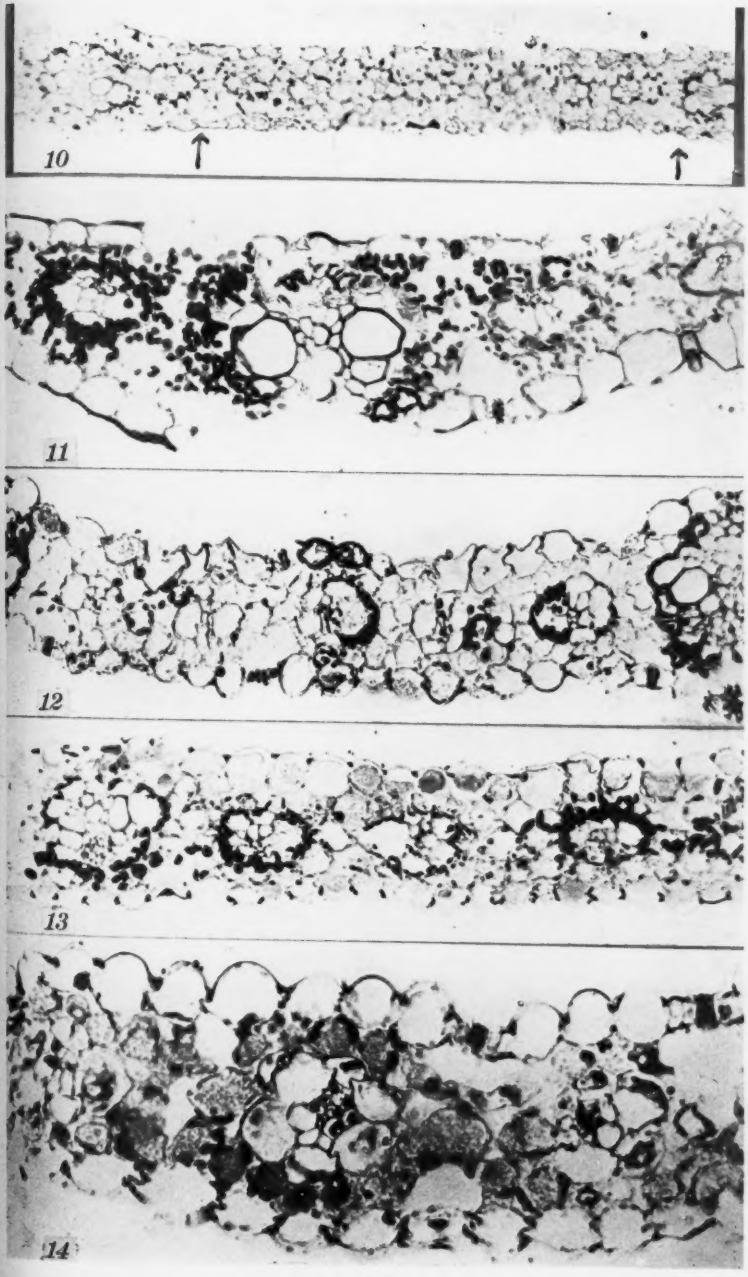


ZIRKLE on ZEA MAYS



ZIRKLE on ZEA MAYS

FIG. 5.—Longitudinal section of root in region of differentiation, showing two sizes of mitochondria; ×800.

PLATE XI

FIG. 6.—Same tissue as fig. 4, older stage; transitional stages plainer here; ×800.

FIG. 7.—Epidermal cells near tip of root showing occasional plastid-like form assumed by mitochondria; ×800.

FIG. 8.—Cross-section of leaf of yellow-green lethal stock; ×250.

FIG. 9.—Cross-section of leaf of second albino stock; probably an extreme case of virescence; ×250.

PLATE XII

FIG. 10.—Cross-section of leaf showing earliest recognizable difference between dark and light areas in maternal inheritance stock; arrows mark limits of white stripe; ×125.

FIG. 11.—Cross-section of older leaf of same stock, showing difference in size of two types of plastids and difference in ability of types to retain stain; ×175.

FIG. 12.—Cross-section of still older leaf, showing disappearance of plastids in lighter region and fuller development of plastids in certain cells; ×175.

FIG. 13.—Cross-section of "argentea" leaf, showing small plastids in minute light green stripe; ×175.

FIG. 14.—Cross-section of leaf in expallescent-virescent plant, showing half-grown plastids and granular cytoplasm; ×200.

MULTIPLE CONES IN ZAMIA FLORIDANA

FRANCES GRACE SMITH

(WITH FOURTEEN FIGURES)

Since a summary of the views concerning the stem development of cycads was included in a former paper (6), it is necessary here only to mention the results of investigations made available since that date. MATTE (5), in the introduction to his monograph, includes a historical sketch of the subject, but in his own investigations seems not to have worked upon the base of the peduncle nor to have considered its origin. CHRYSLER (3), in his study of *Microcycas calocoma* from Cuba, states that cone domes are present; that the cones are single and terminal.

CHAMBERLAIN (1), studying *Macrozamia moorei* from South Africa, finds that ovulate cones, two to eight in number, and staminate cones from twenty to one hundred and three on an unbranched plant, are borne clearly in a lateral position. He considers the stem monopodial in its development, and without cone domes, all cones coming from the axils of leaves, as in Cycadeoidea. In an unpublished paper upon *Bowenia serrulata*, Mrs. ALICE BAILEY shows cones borne on slender branches, the cone arising in the axil of a leaf. It was not possible from the meager material on hand to ascertain whether or not cone domes were present. CHAMBERLAIN'S (2) clear exposition of the presence of cone domes in all stems with a terminal strobilus furnishes a supplementary criterion for a determination of the stem type in the different cycad species. This relation, as well as the average number of cones produced, is shown in table I.

While the species of *Dioon*, *Ceratozamia*, *Microcycas*, *Stangeria*, and *Bowenia* have a single cone as a rule, the species of *Zamia* frequently bear more than one cone and these close to the tip of the stem. When the strobilus is single the method of development of the trunk is clearly sympodial, and the development might in certain cases be rapid enough to bring to maturity several cones in the same season. On the other hand, so many of these cases were found and

the number of staminate cones to a plant was so large that, in view of the situation in *Macrozamia* and *Encephalartos*, it was thought worth while to examine *Zamia* a little more thoroughly as to the origin and direction of the bundles, so that the former statement of the presence of a sympodium might be corrected or emphasized.

TABLE I

Species	Ovulate cones	Staminate cones	Cone domes
Dioon	Terminal, single as a rule	Terminal, single as a rule	Present in both
Ceratozamia	Terminal, single as a rule	Terminal, single as a rule	Present in both
Microcycas	Terminal, single as a rule	Terminal, single as a rule	Present in both
Zamia	Terminal, frequently more than 1, sometimes 6 or 7	Terminal, frequently more than 1, 10 not uncommon	Present in both
Cycas	Not compact, sporophylls making a crown	Terminal, single	Absent in ovulate, present in staminate plant
Bowenia	On slender branches, single	On slender branches, single	?
Encephalartos	Lateral, often more than 1, 3–5 common	Lateral, many	Absent in both
Stangeria	Terminal, single as a rule	Terminal, single, occasionally 2 or 3	Present in both
Macrozamia	Lateral, 2–8	Lateral, many, up to 100	Absent in both

Methods

Of the many plants of *Zamia floridana*, collected and sent from Miami, Florida, fifteen were used for this study, having from two to ten cones each. Four plants bore ovulate cones, two to four on each crown; eleven bore staminate cones, three to ten each. These are about the usual proportions, for the exceptions to the single cone condition are more numerous among staminate plants, and the number of cones is larger.

The plants were cut 3–4 inches below the crown (the leaf blades were trimmed before they were sent), and the petioles and the upper part of the strobili removed, leaving the peduncles and enough of the sporophylls to determine whether they were megasporangia or microsporangia, and whether they were old or young. A diagram of each plant, looking onto the crown, was made to show the point

of origin and the comparative size of the cones. Then serial sections were made, cut longitudinally 2–5 cm. thick, killed in alcohol and formalin-acetic acid, washed, dehydrated, and cleared in xylol with

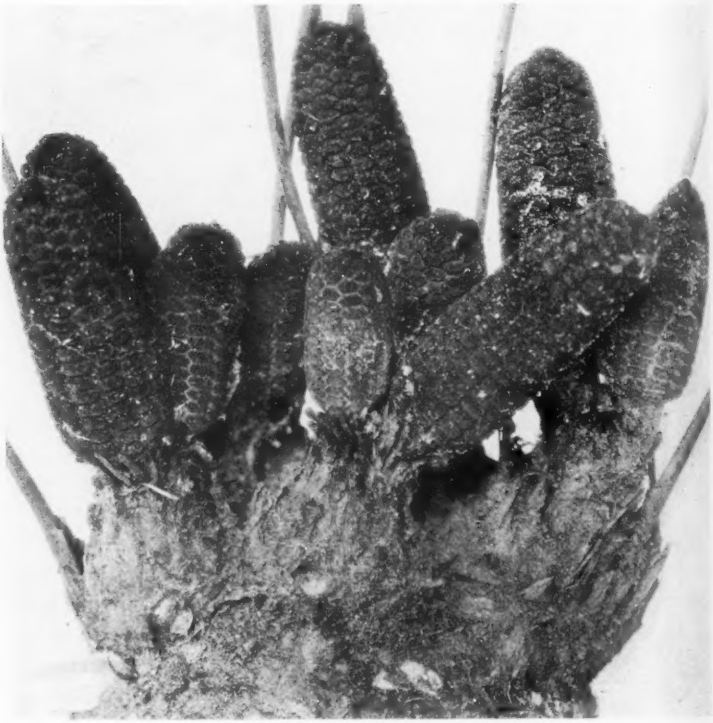


FIG. 1.*—Crown of plant with ten staminate cones, leaf blades and some leaves removed.

as careful a transition between grades of alcohol and xylol as in small specimens. The longer the immersion in xylol the clearer the course of the bundles became. In some cases carbon disulphide was added to the xylol. The sections were studied over an electric light bulb and drawn with a camera lucida.

The cones were all of this year's growth; in most cases even the

* All figures are of *Zamia floridana*.

peduncles of last year's cones were so shriveled that they were hard to recognize. When removing the petioles from the plants before sectioning, it was noted that frequently the compact head of cones resolved itself into a much branched one (figs. 1, 2). Whether this was due altogether to external causes or not it is impossible to say, but fire often sweeps through the open pine forest in which *Zamia*

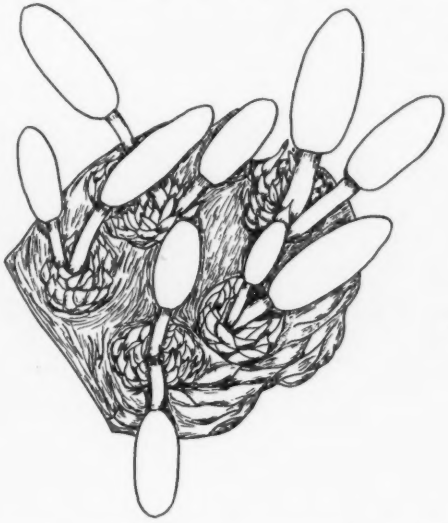


FIG. 2.—Same plant as fig. 1 with scale leaf removed, showing five buds developed from crown; each bud has two cones.

makes a part of the undergrowth, and partially destroys the foliage and probably some of the trunk. Sometimes the land has been roughly cultivated and the plants cut up; sometimes "the practice is to dig only the upper portion of the plant, leaving the tap root which soon produces another harvest for the starch mill." This accounts for the wide branching of *Zamia* in fig. 3, my collector writes. Several branch tips showed young cones when received in August and September.

Of the plants studied, one ovulate and four staminate showed good examples of typical sympodial growth. In the other plants this was not so simple, for at one point at least, in each specimen, there

was a definite forking of the bundles (that is, a lateral direction with reference to the terminal bud). Figs. 4 and 5 show the simple

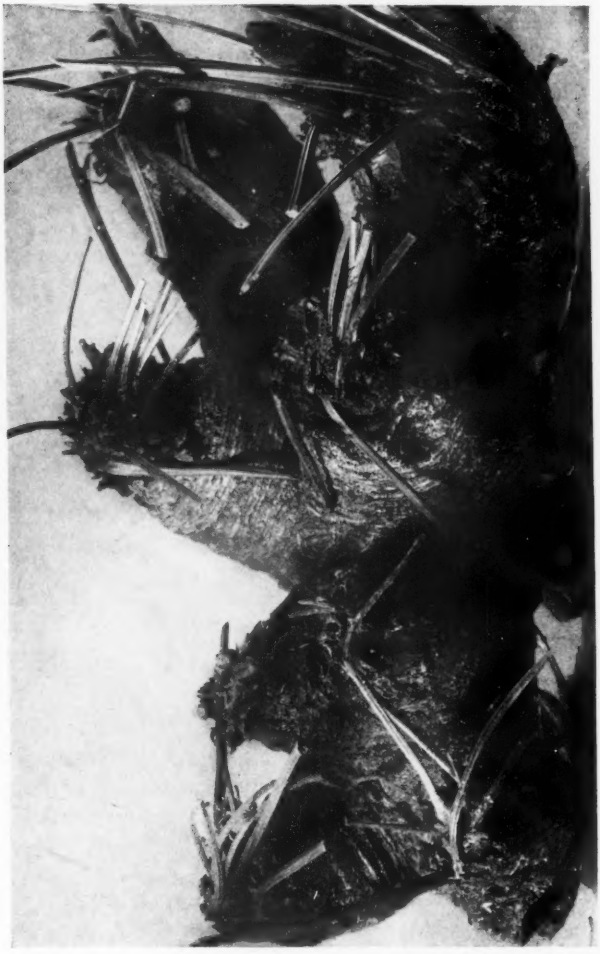
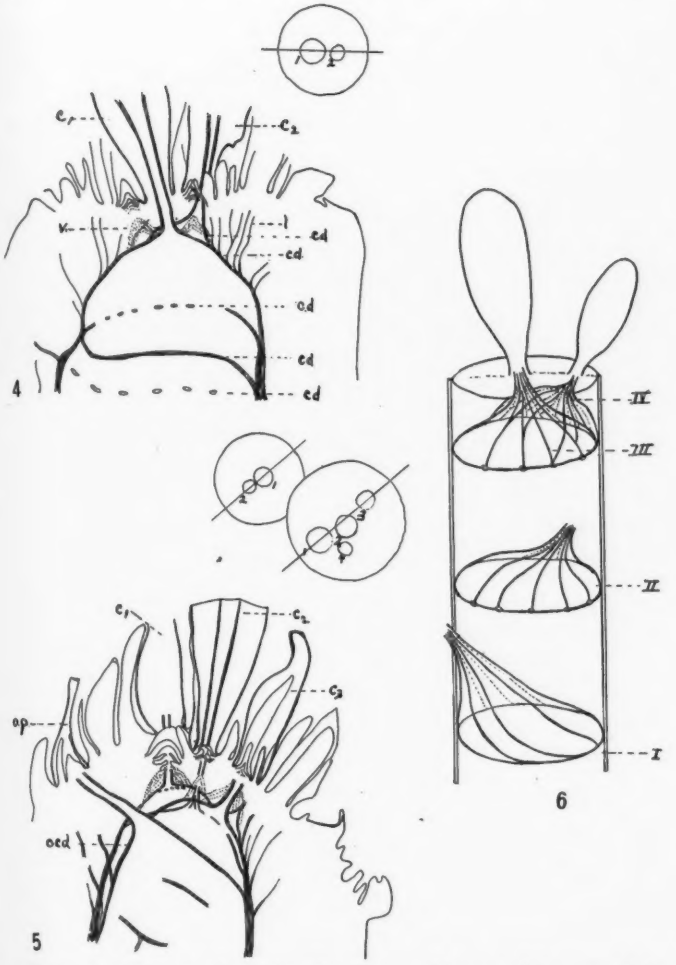


FIG. 3.—Photograph of plant developed after injury to crown

sympodium in an ovulate and a staminate plant respectively, while fig. 8 (staminate) shows a sympodium in each branch. In fig. 5 the



FIGS. 4–6.—Fig. 4, longitudinal section of crown with two ovulate cones and showing a good sympodium (cf. fig. 6); lowest cone dome (*cd*) cut transversely across bundles, as shown in II of fig. 6; next cone lengthwise, as in I of fig. 6; the third transversely again, as in II; the fourth like III of fig. 6; the fifth like IV of fig. 6; *l*, leaf trace; *v*, vegetative point. Fig. 5, longitudinal section of staminate-coned plant: *op*, old peduncle, not visible in crown diagram; *ocd*, old cone bundles; cone numbers agree with those of diagram (note order of formation of cone domes and attachment of bundles of each to preceding cone dome). Fig. 6, diagram of cone dome formation: I, II, III, IV, different views of cone domes.

shriveled peduncle on the left does not show in the plan of the crown; the three next older cone domes are indicated and three vegetative points are developing.

In the cleared sections these can be distinguished by the greater

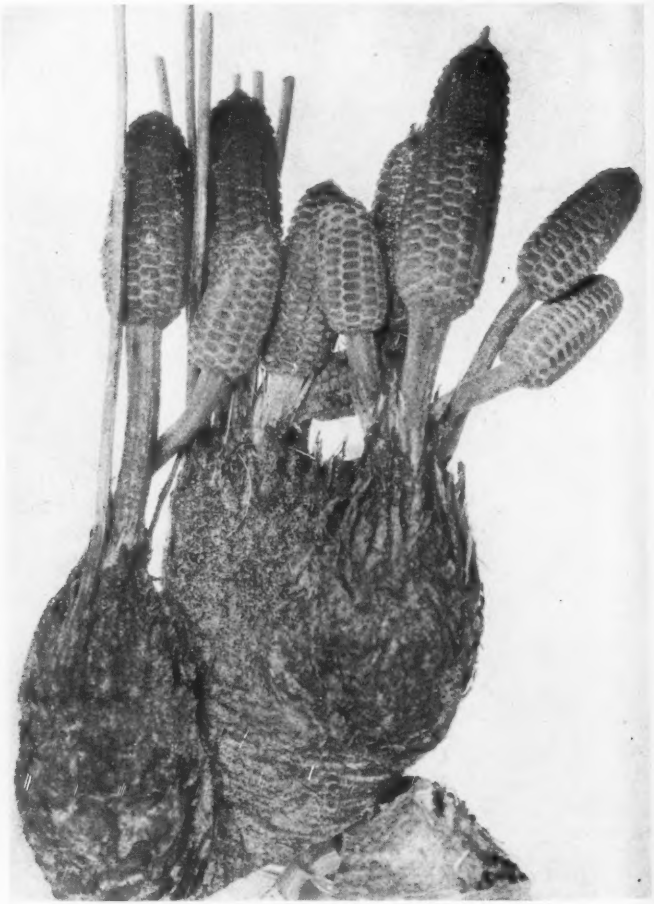


FIG. 7.—Plant with ten staminate cones, two of which are on bud below level of crown.

density of the cells (lesser transparency), and, as indicated in the drawing, they frequently appear close to the converging bundles of the cone next older, rather deeply placed in the tissue. No attempt was made to trace the development of the meristem before the appearance of the bundles in the cone, but their course could be followed back until they fused with older bundles, making a one-sided cap around the cone dome preceding (figs. 4, 5). In many sections the

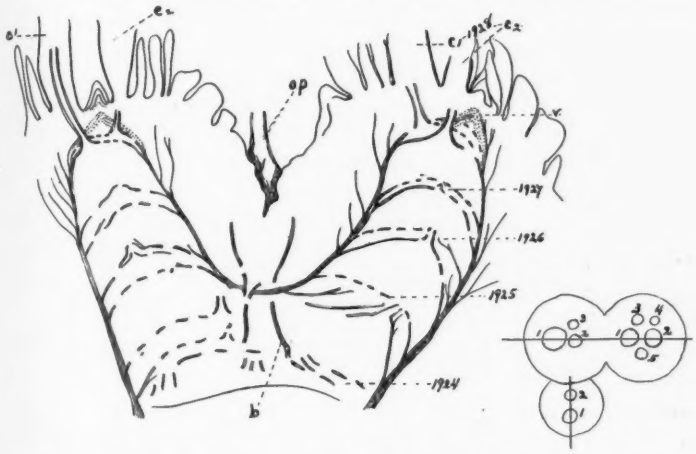


FIG. 8.—Longitudinal section of same plant as in fig. 7 with two branches represented: *b*, bundles to an old peduncle (*op*) which does not show in crown diagram below; two branches develop sympodially with two cones each year as shown by cone domes.

fine veins running to the leaves could be clearly distinguished. In fig. 4 the lowest cone dome was cut transversely across the bundles, the separate bundles showing cross-sections of xylem and phloem. In the next cone dome they were cut lengthwise; the third was transverse; and the fourth showed the origin of the bundles of the oldest cone to be seen on the plant and the arches of this dome made by the bundles as they curve to enter the peduncle. Fig. 6 illustrates this diagrammatically. Fig. 7 (a photograph) and fig. 8 (the longitudinal section) illustrate a plant with ten staminate cones having in each arm or fork a succession of cone domes, and so close together

that the production of multiple cones must have been a constant process in this specimen. The origin of a growing point often appears at right angles to the line of cones, but in the comparatively few plants examined it was impossible to discover that the cones appeared in any regular phyllotaxy.

When the plants showed no forking of the bundles to the cones the number of cones varied from two to six, but a larger number of cones resulted where the "branching" of the bundles was a feature. This is not dichotomy, for between the branches there can usually be seen the vascular supply of an older cone, of which the peduncle may be gone and the tissue piled over the severed point. These so-called branches at their point of origin seem to have no relation to leaves, but to be closely connected to the terminal cone formation as lateral extensions (figs. 9, 10, from the same plant).

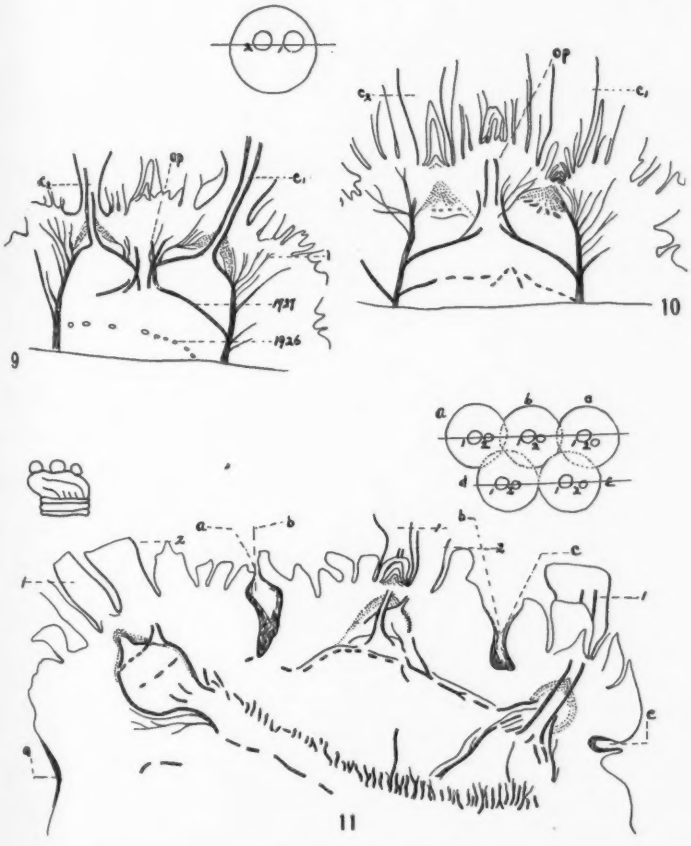
No certain cause for the development of multiple cones is known, and if one refers it to fire or cutting, one only pushes back a little further the unknown stimulus for the development of the cells in the meristematic region which eventually produce the cones.

In five of the specimens which branched there was a depression in the crown over the angle of divergence, and occasionally a discoloration of the tissue, as if some injury had taken place. In a surface view this was seen to be accompanied by an elevation of tissue covered by scale leaves (figs. 1, 2, 11), and frequently undiscovered until these were removed.

There was no suggestion of any injury in four plants, one of which is shown in figs. 12 and 13, so very broad in the crown that the order of development is difficult to follow and only three peduncles showed in surface view. The left-hand bud in the figure seems to have arisen from the cone bundles next to it, but at a later time than the two at the right of the old cone dome.

In two plants or perhaps three, there were suggestions of so much injury that the normal development of cones was interfered with, and the plant had recourse to the formation of adventitious buds, which after a year or more developed cone domes as the parent did (fig. 14). Fig. 7 shows a rounded growth on one side of the trunk, which might have had this origin, but the sections of the plant did not show any connection of the bundles with those of the main trunk,

although the cutting of this bud was at right angles to the main part. If more of the trunk had been left, however, the connection might



FIGS. 9–11.—Figs. 9, 10, two longitudinal sections of same ovulate plant: at center was an old cone as shown by direction of bundles (*op*); growing point is forming almost over it; two cones have their origin close to these bundles; cone domes of 1927 and 1926 show that in those years there was a sympodium. Fig. 11, longitudinal section of plant illustrated in figs. 1 and 2, showing three buds (*a*, *b*, *c*); buds limited to spaces between letters *aa*, *bb*, *cc;* upper left gives diagrammatic view, showing one-sided development of crown, diagram in upper right gives surface view of crown with five buds; cones numbered according to size of each bud; line marks direction in which cuts were made.

have shown. There were two cones on the bud and eight on the trunk.

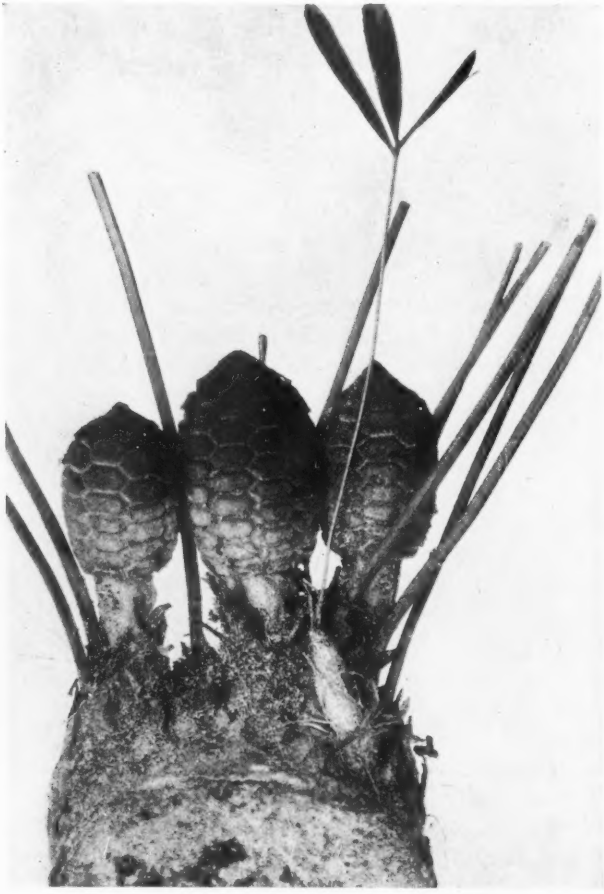
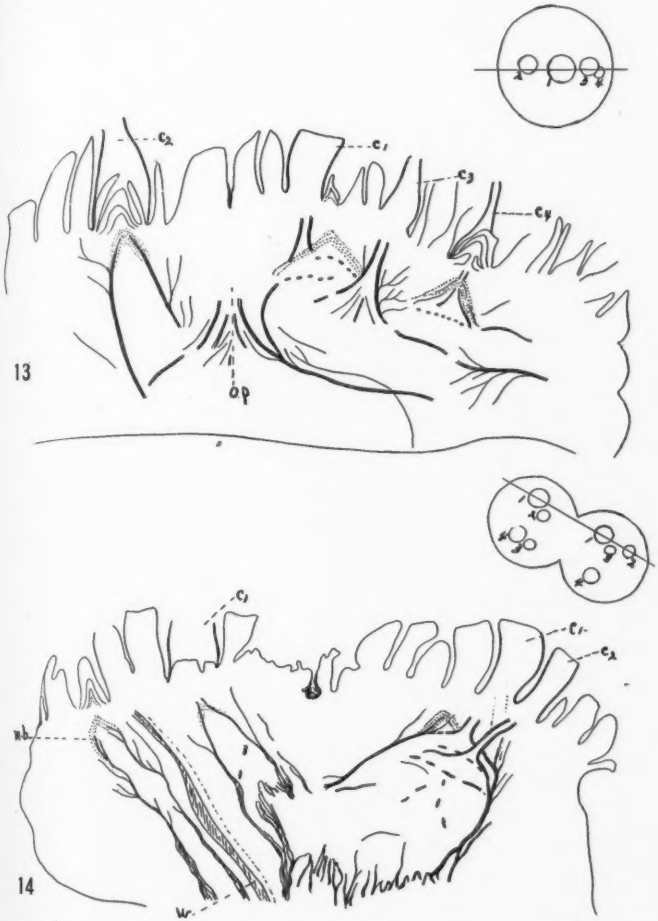


FIG. 12.—Ovulate plant with very broad crown

That adventitious buds develop may be the explanation of the first photograph and the figure accompanying it, which show ten cones. The longitudinal sections could disclose only three of these adventitious buds at a time, but they show the connections of the

cone bundles with the main stelar bundles, and no cone domes appear close to the tips of the branches. COULTER and CHRYSLER (4) record two types of "regeneration" in *Zamia:* (1) adventitious bud-



FIGS. 13, 14.—Fig. 13, longitudinal sections of same plant sympodially developed on right; *op*, old cone bundles; at left of these another cone and growing point developing as lateral offset. Fig. 14, longitudinal section of plant with eight cones; deeply stained streak at left (*w*) made by cut in tissue; bundles at right and left of this crack seem to arise directly from main stelar tissues; *nb*, new bud.

ding, and (2) the actual restoration of lost parts. They find "these new shoots arise most frequently from the vascular part of the central cylinder but also from the peripheral part of the wounded surface of the cortex," i.e. the wound phellogen. In the plant illustrated in fig. 11 there is no evidence of wounding, unless a very one-sided development of the crown may be it. The three pairs of cones are distinctly traced back to the main stelar bundles of the trunk. There was no trace of cone domes in this part, but in the shoot which is very vigorous, on the left in fig. 11, two cones already have formed and the middle pair will show them next season. The very broad crown gives a diagonal section of the stelar region, but the tip is toward the left, so that the other buds are almost lateral in relation to it (fig. 11). In fig. 14, referred to before, the development of a new bud is close to what looks like a long and deep cut in the tissue, but the connections of the buds are with the vascular system at a distance from the vegetative points of the buds, and there is no sign of any relation to the neighboring wound phellogen. MISS STOPES (7) records the development of buds in *Cycas* from leaf bases. It is evident that here we have only the most common type of shoot formation from the vascular part of the central cylinder.

Summary

Multiple cones in *Zamia floridana* may have three methods of development:

1. They may be traced back to a sympodium which shows a rapid formation of cone domes one after another. This is most common.

2. Sympodial development of cone domes is found in certain branches, but in them the forking of the bundle system begins near the base of a terminal cone, which was not turned to one side but remained erect as shown by the remnant of its peduncle.

3. Adventitious buds appear in the cortical tissue closely connected with the stelar system of the trunk, and these buds continue their development like typical stems.

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SWARMING OF DINOFLAGELLATES IN DELAWARE BAY, NEW JERSEY[1]

G. W. MARTIN AND THURLOW C. NELSON

(WITH FOUR FIGURES)

The marked discoloration of extensive areas of ocean water, as well as quiet bodies of fresh water, known as red water, bloody water, milky water, and by similar terms, has been noted since ancient times. The vivid description of the turning of the waters of the Nile into blood, found in the seventh chapter of Exodus, probably refers to something of the sort. Numerous references, including citations from STRABO, TACITUS, and other classical writers, and from a number of navigators of the sixteenth to the early nineteenth centuries, are given by DARESTE (3). The causes to which these appearances have been attributed are various, and sometimes very curious; but for the past hundred years it has been recognized that they are due to the presence in the water of dense swarms of minute and commonly microscopic organisms. These may be blue-green algae, copepods, diatoms, or members of various other groups, but the most striking instances are due to dinoflagellates. CHARLES DARWIN (4) saw extensive areas of such water off the coast of Chile in 1832, and describes the organisms causing certain of them in such a way as to leave no doubt that they were dinoflagellates, although he does not name them. ALLMAN (1) reported an outbreak of brown water in freshwater ponds of the Dublin parks, due to a dinoflagellate which he called *Peridinea uberrima*. CARTER (2) noted such water near Bombay, and attributed it to a form which he named *Peridinium sanguineum*. WHITELEGGE (10) describes an extensive outbreak of red water in New South Wales, due to *Glenodinium rubrum*, which caused great devastation among oysters and other marine animals not able to escape from the area. In his opinion the devastation was due to suffocation caused by the exhaustion of the oxygen in the water, complicated, after the first attack, by the fouling of the water from the decomposition of the dead bodies. NISHI-

[1] Contribution no. 16, New Jersey Oyster Investigation Laboratory. Paper of the Journal Series, New Jersey Agricultural Experiment Station.

KAWA (9) reports an outbreak of red water in Japan produced by *Gonyaulax polygramma*. This did no damage, but earlier outbreaks in the same locality were reported by local residents to have been highly destructive to pearl oysters, fishes, and crustaceans. HIRASAKA (5) reports another case in Japan due to *Gymnodinium sanguineum*, which did no damage, and cites an earlier outbreak reported by OKAMURA (also cited by KOFOID and SWEZY 7) in which some injury to fish was observed. Perhaps the most striking examples are those reported by KOFOID (6) from coastal waters of California, due to *Gonyaulax polyhedra*, where in some cases the discoloration was many miles in extent, and was accompanied by great devastation among the bottom-living animals. KOFOID and SWEZY mention an outbreak of yellow water, due to *Gymnodinium flavum*, and accompanied by a great display of luminescence.

A similar condition seems to be of rather common occurrence in parts of Delaware Bay, New Jersey, especially in the area lying to the east and northeast of Deadman Shoal, Maurice River Cove. Through the kindness of Mr. J. RICHARDS NELSON, in charge of oyster investigations of the New Jersey Agricultural Experiment Station and Board of Shell Fisheries in Maurice River Cove, we have been able to observe a number of striking exhibitions of red and brown water during the late summer of 1928.

On July 30, at 8:00 A.M., with the tide at approximately one hour ebb, a belt of brown water was seen along the shore below Dias Creek, Cape May County. This brown belt extended approximately 7 meters out from the shore to a depth of 2 meters, where it met in a very definite sharp line the gray-brown water of the bay. With but slight variation in width, it stretched up and down the beach as far as the eye could distinguish it. The sky was clear, and a gentle south-southwest wind stirred the surface. At low tide the broad flats of sand and mud ran bare, leaving sloughs with water a few centimeters deep. This water, exposed to the direct rays of a hot sun, continued to show the same brown color, resembling weak tea.

Examination of the water showed it to be swarming with a small, yellowish green, fusiform dinoflagellate, which belonged to an apparently hitherto undescribed species, and which has since been described (8) under the name *Amphidinium fusiforme*. The organisms

were exceedingly active for a few minutes under the microscope, finally coming to rest and almost immediately disintegrating.

As the flood tide came in over the flats, the brown water became mixed with the incoming waters of high turbidity and the brown color disappeared. Oysters which had been exposed during low tide were opened after the water had risen half a meter over them and their stomach contents immediately examined. These were found to consist of a veritable soup of disintegrating dinoflagellates, chiefly *Amphidinium fusiforme*, with numerous specimens of the much larger *Gymnodinium splendens*.

On August 2 at 10:00 A.M., red water was observed over the oyster grounds of Inner Deadman Shoal, in water approximately 6 meters deep. The tide was high, beginning ebb, with a hot sun and a light west wind. Three belts of color were seen, each 3–5 meters broad and extending for several hundred meters. In hue the water varied from that of weak cocoa to a distinct blood red, as though great quantities of blood were being mixed with the sea water. The water in the belts had a temperature of 25.0° C., pH 8.2 with Cresol red, and a specific gravity of 1.01614 (corrected to 17.5°C.). Water just outside the belts had the same temperature, but a pH of 8.15 and specific gravity of 1.01610. A sample of the red water observed under the microscope showed enormous numbers of *Amphidinium fusiforme*, together with numerous other dinoflagellates. In watching the belts from the deck of a boat, it seemed as though a given spot varied in intensity, thinning out to the hue of weak cocoa and then quickly increasing to a blood red color.

On August 24 at 11:40 A.M., with the tide 2 hours' ebb, a belt of red water some 20 meters wide and several hundred meters long was encountered over the oyster beds of Inner Deep Water. The sun was shining brightly, and short choppy waves 1–2 feet high were running against the tide, under a moderate south wind. As the waves passed through the belt they became partially flattened out, as though in an oil slick.

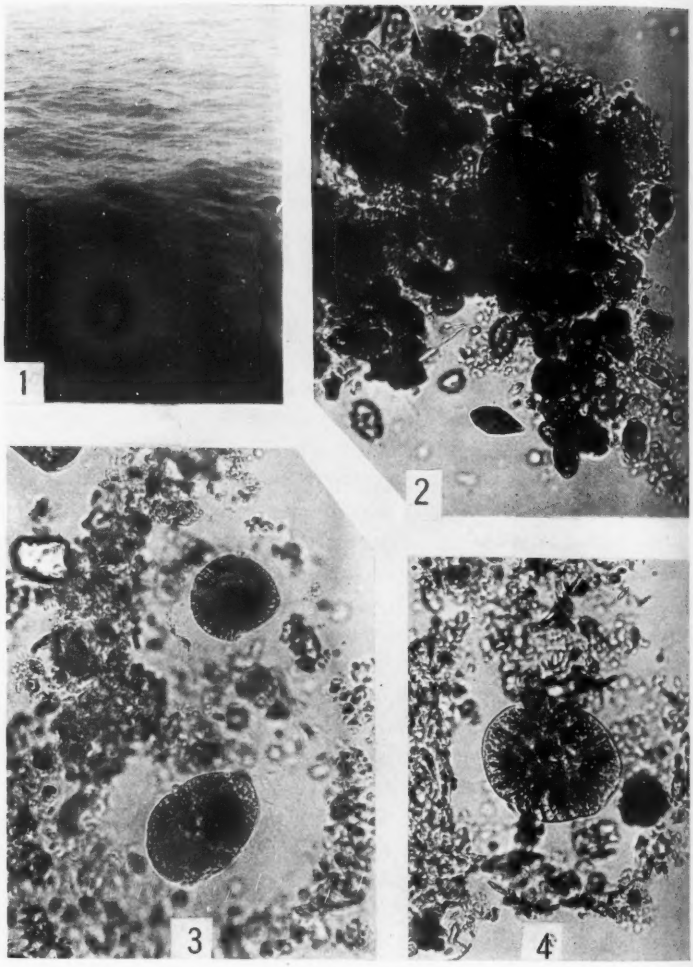
On August 30, about midday of a hot clear day, a large number of patches and belts of red water were observed. The tide was about half ebb, the sea fairly calm, and the sunlight intense. The temperature of the water varied from 28.6 to 28.8° C. The patches varied in color from chocolate brown to deep blood red, and in area from a

few square rods to many acres, being mostly rather long and narrow. One such patch could be observed from the roof of a cabin, perhaps 8 feet above the surface of the water, to extend for at least a mile in either direction. The line of demarkation between the red water and the clear green normal water was sharp and distinct, as the accompanying photograph (fig. 1), snapped with an ordinary pocket camera without a filter, demonstrates. Examination of plankton samples from various patches of red water showed that in most cases *Amphidinium fusiforme* was the dominant species present. Associated with it in large numbers was a *Gymnodinium* agreeing closely with *G. uberrimum* (Allman) K. & S. as described by KOFOID and SWEZY (7). Reference to ALLMAN'S original paper (1), however, raises a question as to whether ALLMAN'S *Peridinia uberrima* really is, as KOFOID and SWEZY claim, identical with the form they call *Gymnodinium uberrimum*. It seems better, therefore, not to attempt to apply the specific name at this time.[2] In a few samples the *Gymnodinium* was the dominant species. Associated with these two forms were numerous other dinoflagellates, particularly *Gymnodinium splendens*, *Polykrikos kofoidi*, and species of *Gonyaulax* and *Peridinium;* also diatoms, particularly *Sceletonema costatum* and species of *Coscinodiscus*, *Biddulphia*, and *Chaetoceros*.

The contrast between the yellow-green color of *Amphidinium fusiforme* and the yellow-brown color of the associated *Gymnodinium* (as seen under the microscope by transmitted light) on the one hand, and the red and reddish brown color of the patches of red water in which they occur on the other, is very significant, suggesting strongly that these densely packed masses of chlorophyll-containing cells make of the surface layers of the water essentially a nearly continuous suspension of chlorophyll, and that the reddish color is nothing other than the well-known reddish fluorescence of chlorophyll. This is borne out by the fact that the red shades predominate in the deeper water, while where it is shallow enough to permit light to be reflected from the bottom, through the organisms, the brownish color predominates.

What causes dinoflagellates to mass together in this way has

[2] Since this manuscript was submitted, the larger *Gymnodinium* referred to has been described (Univ. Ia. Studies Nat. Hist. 12:16. 1929) as *Gymnodinium subrufescens* Martin.



FIGS. 1–4.—Fig. 1, margin of patch of red water showing sharp delimitation of area; Maurice River Cove, August 30, 1928. Fig. 2,* photomicrograph of plankton from patch similar to that illustrated in fig. 1, and collected at same place and date; mass of *Amphidinium fusiforme*, together with colloidal detritus and a few diatoms, collected about a cell of *Gymnodinium;* killed in osmic acid, followed by chrom-acetic solution, then formalin, and stained in Trypan blue. Figs. 3, 4, cells of *Gymnodinium*, showing thick, hyaline gelatinous envelope, margin of which is outlined by clinging detritus; killed in saturated solution of bichloride of mercury, followed by formalin, and photographed unstained; Deadman Shoal, August 29, 1928.

* Figs. 2, 3, and 4 photographed at magnification of approximately 800 diameters and reduced one-fifth in reproduction.

never been explained, but observations made upon this occasion may have some bearing upon the matter. Red water plankton, in which the *Gymnodinium* predominated, when killed with a strong solution of iodine in potassium iodide and examined microscopically, showed each cell of the *Gymnodinium* to be surrounded by a gelatinous envelope about as thick as the diameter of the cell itself, so that the water in which they occurred must have been a nearly continuous mass of soft jelly. This gelatinous envelope is also visible in material killed with a saturated solution of bichloride of mercury, although it is not quite so apparent, since neither the jelly nor the cells are stained by this fixative (figs. 3, 4). It is less regularly seen in material killed by osmic acid and then transferred to chrom-acetic solution, and never in any of the other reagents used. Gelatinous envelopes are common among dinoflagellates when encysted, but not when active. The cells referred to in this connection were actively motile. A similar envelope has been noted occasionally surrounding other species of naked dinoflagellates in the active condition, but only when killed by the iodine or bichloride methods. In red water plankton in which *Amphidinium fusiforme* is the dominant species, the *Amphidinium* cells tend to cling together in clumps, but no gelatinous envelope can be demonstrated. In many of the clumps (although not in all), however, they may be seen to be clustered thickly about a cell of the *Gymnodinium* (fig. 2). This gelatinous envelope may well be a factor of importance in holding the organisms together, once they are massed by a favorable combination of light, water temperature, and tidal currents.

In no case was there any suggestion of injury to fish or other marine animals in connection with the swarming. On the contrary, MR. NELSON reports that it is just those areas in which red water is most frequently encountered that have been found by long experience to provide the best fattening grounds for oysters in the entire district. Experience elsewhere, as shown by the references here cited, suggests that these dense swarms may constitute a potential menace to shellfish and to other marine animals. Probably the relatively swift tide, and the comparatively small area occupied by the red water in Delaware Bay, render the possibility of damage to oysters in that region rather remote.

Summary

1. Instances of red water occurring in Delaware Bay and due to the swarming of *Amphidinium fusiforme* and other dinoflagellates are described.

2. It is suggested that the characteristic color of the water which gives the phenomenon its name is due to the reddish fluorescence of the chlorophyll present in such great quantities.

3. A factor tending to hold the cells together in dense masses, once they are brought together, may be the gelatinization of the outer envelope of the active cells, not heretofore noted in this connection.

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CURRENT LITERATURE

BOOK REVIEWS

Enzymes

Biological investigators will welcome the recent translation of WALDSCHMIDT-LEITZ'S[1] book on enzymes. The author attempts to give a concise account rather than an exhaustive treatise of the main principles of enzyme chemistry, and the special characteristics of the most prominent enzymes. The viewpoint is essentially that of the WILLSTÄTTER school, and many of the results of WILLSTÄTTER and his co-workers, which have recently been published,[2] are summarized in the book. This is only natural, since WALDSCHMIDT-LEITZ is an active associate of WILLSTÄTTER. The book is not merely a summary of the results obtained by the WILLSTÄTTER school, however, but is intended as a general summary of enzyme literature. It is a good volume from which to get a review of the work of foreign investigators, especially the German workers. One feels that the work of English and American scientists has been neglected, although this is perhaps to be expected in a work of this kind. The translator has materially extended the original German text, the additions, however, being mainly according to the viewpoint of the WILLSTÄTTER school.

The book is divided into two main parts. The first is a general section, with chapters on development of the ferment concept, enzymes as colloids, enzymes as electrolytes, enzymic kinetics, enzymic reaction systems, enzymic specificity, and detailed procedures in preparative enzyme chemistry. One of the main contributions of WILLSTÄTTER and his co-workers has been the devising of methods of obtaining pure enzyme preparations. These methods are rather fully outlined. In the second section the main enzyme systems are treated. There are chapters on esterases, proteases and peptidases, aminoacylases, carbohydrases, catalases, peroxydases, oxydases, and fermentation enzymes. In the chapter on the aminoacylases, urease is treated, and SUMNER'S investigation which resulted in the isolation in the crystalline state of a protein of the globulin group, identical with urease, is referred to. It is interesting to note that the author states that the German workers, EULER and BRUNIUS, in their recent investigation have been unable to confirm SUMNER'S findings. SUMNER attributes their failure to a low urease content in the material used. It would seem that some such explanation for their failure must be true, for SUMNER'S work

[1] WALDSCHMIDT-LEITZ, ERNEST, Enzyme actions and properties. Transl. by ROBERT P. WALTON. pp. xv+255. New York: John Wiley & Sons. 1929.

[2] WILLSTÄTTER R., and coworkers, Untersuchungen über Enzyme. vols. 2. pp. xvi+1775. Berlin: Julius Springer. 1928.

seems rather definitely to prove that the urease of the jack-bean and the crystalline protein isolated from the jack-bean are identical.

One of the main values of the book is that it gives an authoritative modern viewpoint of enzyme chemistry. This is very much needed, for much of the older literature on enzymes is conflicting and therefore worthless for arriving at a true conception of the nature of enzymes and their action. For example, it is a common statement in the literature that some enzymes are given off first in the cell in an inactive state called "zymogen" or "proenzyme," but the author states that this viewpoint has not been proved. The enzyme merely appears to be in the inactive form because some condition for its activity, very commonly the pH, is not right. While WILLSTÄTTER has not perhaps been as successful in elucidating enzyme chemistry as he was in determining the chemical constitution of chlorophyll and other complex organic compounds, yet his contributions to our knowledge of enzymes have been many. A book that summarizes some of the results obtained by this master of organic chemistry and gives his viewpoint is especially to be welcomed.—S. V. EATON.

A new state flora

SCHAFFNER, who has seen many years of service at the Ohio State University, has produced a field manual of the flora of Ohio and adjacent territory.[3] The book is attractively bound, and, from its small dimensions, merits the name pocket manual. A formal and full phyletic system is introduced for the "complete classification into phyla, classes, subclasses, orders, and families." In many of the larger families and genera, condensed synopses are given. The numerous keys, based as they are upon floral groups unlike those of any other one area of similar size, afford many fresh viewpoints as to diagnostic characters, and will doubtless find use at the hands of students in neighboring states. The author acknowledges help from a long list of his past students who have studied special groups of the Ohio plants. The nomenclature follows the American Code, and is essentially in agreement with that of the second edition of Britton and Brown's *Illustrated flora*. A partial adaptation to GRAY's Manual (7th edition) is afforded by means of generic synonyms and occasional specific synonyms. These latter are far from complete (cf. *Prunus virginiana* and *P. nana*, p. 305; *Vaccinium angustifolium*, p. 391), leaving the work less useful for class work where a correlation with GRAY's Manual is desired. Furthermore, the number of common names for each species is reduced, in the interest of nomenclatural clarity, to one for each species. It remains to be seen whether a state flora will be generally recognized as the proper agency for the introduction of such an innovation, desirable as this might seem to some workers.

Many of the treatments differ from what might be expected. This appears due in some cases to the author's personal views, in others to his failure to ac-

[3] SCHAFFNER, JOHN H., Field manual of the flora of Ohio and adjacent territory. 16mo. pp. 638. Columbus, Ohio: R. G. Adams and Co. 1928.

cept the findings of various workers outside his own state. Thus (p. 357) the name *Quercus rubra* L., which really applies to a tree of the southern and southeastern United States, is retained for the red oaks (*Q. borealis* Michx.) of Ohio; (p. 501) *Xanthium americanum* Walt., a name of very dubious application, is permitted to displace *X. chinense* Mill., and *X. canadense* Mill. is erroneously cited as a synonym for *X. pennsylvanicum* Wallr.; (p. 520) the name *Bidens trichosperma* (Michx.) Britt. is retained despite the precedence of the name *B. coronata* (L.) Britt.; (p. 558) the old and outgrown theory of FRIES, KOCH, and certain others, of the oneness of all dandelion species is readopted, at least in part, making our red-seeded dandelion (*Taraxacum laevigatum* Willd.) and our brown-seeded species (*T. vulgare* Lam.) one and the same.

Numerous typographical errors, especially in the spelling of scientific names, occur to mar the appearance of the book. One notes also various other errors, such, for example, as the omission of *Papyrius* from the main text and index although it is included on p. 583 in the key to Ohio woody plants. The reviewer is led to hope that a second edition may be preceded by a careful revision in these respects.—E. E. SHERFF.

Evolution of genetics

More than success has been attained by OEHLKERS in his recent volume.[4] He set out to give a sketch of the development of plant genetics during the past fifteen years; he has in fact retraced the significant steps in this field since the rediscovery of Mendel's laws. This little book is more than a sketch of the past, it orients critically, and looks into the probable future of genetics. This comprehensive presentation is especially useful and timely, because the enormous and rapid development of the subject has tended to isolate the specific problems and investigators, so that they and their problems get out of touch, not only with the rest of the field of genetics, but even more seriously with the larger botanical problems. The author points out that genetics stands at the turning point. The first period, the analytical, which was characterized by the study of the delineation and transmission of factors, is approaching the end of its domination in genetics, even though much more analytical work remains to be done; and the second period, the synthetic, is already under way. The latter will concentrate more and more on the relationship of genetics to developmental physiology and morphology. Granted the genes, there still remains the greater problem of what lies between transmission of the genes to the zygote and their expression in all the various stages of the developing and mature organism. Another important question is the precise significance of the genes in evolution. In going back more than fifteen years the author is no more than just to his subject matter, for the problems which have become acute were sensed in the

[4] OEHLKERS, FR., Erblichkeitsforschung an Pflanzen, ein Abriss ihrer Entwicklung in den letzten 15 Jahren. Wiss. Forschungsberichte. 18. pp. 203. Dresden and Leipzig: Theodor Steinkopff. 1927. $14.50.

early days of genetics, but were neglected or ignored in the progress of factorial analysis. Some of these problems are stated as chapter and paragraph headings in the book.

The subject matter is discussed under two main headings, hybridization and mutation. Under the first heading, the general part takes up (1) Mendelian rules, (2) cycles of development (life cycles), (3) nucleus and heredity, (4) and (5) chromosomes and heredity, (6) protoplasm and heredity; while the special part takes up (1) sterility and lethality, (2) sexuality, and (3) significance of hybridization in species formation. Under the second heading the following topics are discussed: (1) concept of mutation, (2) factor mutants, and (3) genom mutants. Each chapter is concluded with a bibliography. The volume is well and clearly written.—G. K. K. LINK.

NOTES FOR STUDENTS

Ch'orophyll.—The most significant experimental contribution that has recently appeared is a paper by EMERSON[5] on the chlorophyll content and rate of photosynthesis. The methods of WARBURG have been used in this investigation, and the results are as valuable as those obtained by him in his work on the respiration enzyme. Optical methods were used for estimating the amount of chlorophyll, while WARBURG's methods were used to measure the amount of oxygen liberated. By a careful study of the "photochemical reaction" and the "Blackman reaction" (the ordinary chemical reactions involved in plant growth), EMERSON has concluded that chlorophyll plays some other part in living processes in the plant, besides its rôle in the photochemical reaction.—F. M. SCHERTZ.

Chromosome numbers in Oenotheraceae.—*Circaea lutiliana*, *C. alpina*, and *C. intermedia* have been recently studied.[6] In all of these the diploid number is twenty-two and the haploid eleven. The rather plump chromosomes of the first-named species sometimes show a cross constriction. The other two species have more slender chromosomes, and the constriction is often so deep as to give the appearance of division of one chromosome into two. The author, quoting observations of recent writers, gives the haploid chromosome number for other genera of Oenotheraceae as follows: *Oenothera* 7, *Eucharidium* 7, *Godetia* 7 (and 9), *Jussieua* 8, *Clarkia* 9, *Lopezia* 11, *Fuchsia* 11 (and 14), *Epilobium* 18, and *Chamaenerion* 18.—FRANCIS RAMALEY.

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[5] EMERSON, R., Chlorophyll content and rate of photosynthesis. Proc. Nat. Acad. Sci. **15**:281–284. 1929.

[6] UDDLING, ÅKE, Die Chromosomenzahlen von drei Circaea-Arten. Hereditas **12**:294–296. 1929.